Zootaxa 4021 (2): 243–281
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.4021.2.2
http://zoobank.org/urn:lsid:zoobank.org:pub:DB2A11EF-A092-44C2-9E93-C813FFC324F4

A review of *Galaxiella pusilla* (Mack) (Teleostei: Galaxiidae) in south-eastern Australia with a description of a new species

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Abstract

The dwarf galaxias, *Galaxiella pusilla* (Mack), is a small, threatened freshwater fish from coastal south-eastern Australia. Recent genetic studies, using multiple nuclear and mitochondrial DNA markers, found substantial differences between populations in western Victoria and south Australia ('west region') compared to eastern Victoria, Flinders Island, and Tasmania ('east region') that suggest the presence of a cryptic species. Morphological measurements and meristic counts from multiple populations within each region were undertaken to investigate potential differences between regions. Several characters, found to discriminate between individuals in the regions and to be diagnostic for two taxa, were used to describe a new species, *Galaxiella toourtkoourt*, for the west region. This is only the second species in the Galaxiidae to exhibit sexual dimorphism. The original description of *Galaxiella pusilla*, based on five specimens, is revised following examination of a large number of individuals. Both species are considered nationally threatened and are categorised as 'endangered'; the revised distribution of *G. pusilla* s.s. is reduced by approximately 60%. A number of inconsistencies in the most recent revision of the genus *Galaxiella* are also corrected.

Key words: taxonomic revision, dwarf galaxias, sexual dimorphism, threatened species, freshwater fish, cryptic species

Introduction

Within the Southern Hemisphere temperate family Galaxiidae, the genus *Galaxiella* currently includes three small, freshwater dependent species, namely: *Galaxiella pusilla* (Mack, 1936), *Galaxiella nigrostriata* (Shipway, 1953) and *Galaxiella munda* McDowall, 1978. The geographic distribution of *G. pusilla* is restricted to coastal south-eastern Australia, including Tasmania, while *G. nigrostriata* and *G. munda* are restricted to coastal south-western Australia. *Galaxiella* are distinguished from the other genera of Australian galaxiids by: a) their small maximum size, b) dorsal fin origin distinctly behind the anal fin origin, c) strongly developed caudal peduncle flanges with few procurrent rays, d) lack of laterosensory pores beneath the lower jaw, e) less than 16 principal caudal rays (usually 13–14), f) a rounded caudal fin, g) slender, non-flattened, spike-like caudal neural and haemal spines, and h) 3–4 branchiostegals (McDowall 1978a; McDowall & Frankenberg 1981). Another diagnostic feature of *Galaxiella* is the presence of horizontal stripes along the sides of the body, with juveniles having a crenulated stripe along the lower sides that only persists in adult *G. munda* (McDowall 1978a).

Genetic and morphological studies suggest that the Australian *Galaxiella* are closely related to the South American *Brachygalaxias*—sharing an ancient Gondwanan ancestry (Waters *et al.* 2000; McDowall & Waters 2004). In addition to their comparatively small size, *Brachygalaxia*s and *Galaxiella* are unique within the Galaxiidae for their longitudinal body stripes (including bright yellow to orange-red), and fleshy ventral keel which may play a role in targeted egg placement during mating (McDowall & Waters 2004). Waters *et al.* (2000) estimated the time of separation between *Galaxiella* and *Brachygalaxias* lineages to be around 8.6–30.0 Ma,

Accepted by R. Pethiyagoda: 3 Aug. 2015; published: 25 Sept. 2015

although more recent estimates with multiple genetic markers indicated the time of separation was much earlier, with a mean age between 55.8–58 Ma (Burridge *et al.* 2012; Unmack *et al.* 2012). The divergence time between *Galaxiella* from south-eastern Australia and those in south-western Australia has been estimated to be a mean of 34.3 Ma (Unmack *et al.* 2012) and 28.3 Ma (Galeotti *et al*. 2015).

The dwarf galaxias (or eastern little galaxias), *Galaxiella pusilla*, is of national conservation significance in Australia, listed as 'vulnerable' under the national *Environment Protection and Biodiversity Conservation Act* 1999 and the IUCN Red List of Threatened Species (Saddlier *et al*. 2010). *Galaxiella pusilla* was first described in 1936 with specimens collected from Cardinia Creek, east of Melbourne, Victoria (Mack 1936). Originally placed in the genus *Galaxias* Cuvier, it was subsequently placed into *Brachygalaxias* by Scott (1942), who recognised a number of shared characters between it and the South American species, though this was later regarded as inappropriate (McDowall 1973). Recognising that *Galaxias pusilla* Mack, and the more recently described *Galaxias nigrostriatus* Shipway, formed a species group along with a new species being described, McDowall (1978a) proposed a new genus *Galaxiella* for the three species. Based on morphological and genetic similarities, Waters *et al.* (2000) subsequently recommended that the Australian species of *Galaxiella* be restored to *Brachygalaxias*. However, following a detailed morphological study, the status of *Brachygalaxias* and *Galaxiella* as separate, monophyletic genera was supported, each diagnosed by distinctive, derived characters (McDowall & Waters 2004).

There are substantial genetic differences between eastern and western populations of *Galaxiella pusilla* (Coleman *et al.* 2010; Unmack *et al.* 2012; Coleman *et al.* 2013). Within coastal south-eastern Australia, eastern populations occur from the Mitchell River Basin near Bairnsdale, west to Dandenong Creek near Melbourne, Victoria, including Flinders Island in Bass Strait and north-eastern and north-western Tasmania (Saddlier *et al.* 2010). Also within coastal south-eastern Australia, the western populations occur from the upper Barwon River system near Barwon Downs, Victoria, west to the Cortina Lakes, near the Coorong, South Australia (Saddlier *et al.* 2010). Coleman *et al.* (2010) proposed that *G. pusilla*, as it is currently recognised, may comprise two distinct taxa —based on a mean COI divergence (Kimura 2-parameter distance) of 8.43% between populations in the east and west regions, compared to a mean within-genus COI divergence of 9.93% amongst 207 Australian fish species (Ward *et al.* 2005). In addition, Unmack *et al.* (2012) found fixed differences at eight allozyme loci between eastern and western *G. pusilla* populations, and reported mean cytochrome *b* *p*-distances of 9.1% between eastern and western *G. pusilla*, compared to *p*-distances ranging from 15.4–22.4% between *Galaxiella* species and 11.0% between *Brachygalaxias* species. Congruence between multiple nuclear and mitochondrial markers for an eastern and western lineage supports the notion that *Galaxiella pusilla sensu lato* (s.l.) currently exists as an unresolved species complex of one described species and one candidate taxon (*sensu* Vences *et al.* 2005).

Herein we demonstrate that the two taxa proposed within *Galaxiella pusilla* s.l. can be diagnosed from each other using morphological and meristic characters, supporting their status as distinct species, and describe as a new species the western lineage. Given that the original description (Mack 1936), referable to the eastern lineage, was based on a small number of specimens and subsequent revisions (McDowall 1978a; McDowall & Frankenberg 1981) were also based on a small number of specimens (15 for morphology, 8–48 for meristics), we also redescribe *Galaxiella pusilla* (Mack). Habitat information for both lineages has also been incorporated into the species descriptions. The separation of this currently recognised threatened taxon into two distinct species with smaller geographic ranges has significant implications (Coleman *et al.* 2010; Coleman *et al.* 2013), and we discuss issues surrounding their conservation status and management.

Material and methods

Species concept. A total-evidence approach was adopted to determine the validity of splitting *Galaxiella pusilla* s.l. based on differences in morphology, multiple, conserved, nuclear markers and phylogeny as assessed using multiple mitochondrial DNA gene sections. This is consistent with the 'unified species concept' described by de Queiroz (2007) that considers species as separately evolving metapopulation lineages and incorporates a range of criteria drawn from other species concepts to diagnose species. Similar to the *Galaxias olidus* complex (Adams *et al.* 2014; Raadik 2014) these two *Galaxiella* taxa also meet the criteria for species under the morphological and phylogenetic species concepts (e.g. Turner 1999).

Specimen collection. Specimens in various states of preservation and storage can reduce the accuracy of morphometric measurements (e.g. McDowall & Wallis 1996; Raadik 2014), particularly in diminutive species where measurement error may already be higher than usual. Therefore, all specimens used in this study for morphometric analysis were recently collected by the first author and preserved in a consistent manner. *Galaxiella pusilla* s.l. is predominantly an annual species (Massola 1938; Humphries 1986; Pen *et al.* 1993; Romanowski 2004; Coleman *et al.* in review), therefore specimen collection was made over a short period to minimise potential morphological differences associated with different life history stages. Specimens were collected over a five-week period between 27th June and 2nd August 2013 using dip nets and targeting shallow (<1 m) vegetated margins of habitats from 16 sites that spanned much of the known distribution of eastern and western regions (Table 1). Historical specimens collected from geographic extremes in Tasmania and South Australia between 2008 and 2010 were also included (Coleman *et al.* 2010; Coleman *et al.* 2013), however due to preservation in 100% ethanol for genetic studies and associated shrinkage, these specimens were only used for meristic analysis and no length data is presented. Additionally, the holotype, four paratypes and 20 museum specimens collected from the Grampians region in Victoria in 1996 were included for vertebral counts (see 'material examined' sections within species descriptions and Table 1 for specimen details). Due to the substantial shrinkage and warping of the *Galaxiella pusilla* s.s. holotype that made morphometric measurements unreliable, only meristic data is presented for that specimen.

Because *Galaxiella pusilla* s.l. is sexually dimorphic based on colour, size and shape (McDowall 1978b), an even ratio of sexes was sought, with a total of 20 individuals (males and females combined) collected from most sites, i.e. overall total of 123 individuals from the west region and 163 individuals from the east region. Fish were euthanized using a lethal dose of clove oil prior to fixation in 10% formalin in the field. Following fixation for at least three days, specimens were transitioned to a 70% ethanol solution for longer term preservation by exposure to progressively higher concentrations of ethanol over five days (30% and 50% for two and three days respectively).

Morphometric and meristic measurements. Morphometric and meristic characters were mostly measured or counted following the procedures described by Hubbs & Lagler (1958) which have been modified for, and include additional measurements found useful in, other galaxiid taxonomic studies (McDowall 1970; McDowall 1978a; McDowall & Frankenberg 1981; McDowall & Wallis 1996; Raadik 2014). Many of these characters and the method of measurements have been recently re-defined by Raadik (2014). With exception of vertebrae, all measurements and counts were conducted using a Nikon SMZ800 dissecting stereomicroscope on adult fish (total length (TL) 19 mm or greater). Total length and standard length (SL) measurements were taken to the nearest 0.01 mm with digital display vernier calipers, while other morphometric measurements were made with an eyepiece micrometer for improved accuracy with smaller features. All measurements and counts were conducted on the left hand side (LHS) of specimens, except for gill rakers on the first branchial arch which were counted on the right hand side (RHS), to avoid physical damage to the LHS (see below). To increase the accuracy of gill raker counts, particularly small rudimentary and vestigial rakers, the entire first gill arch was carefully removed (by gently pulling towards the snout to reduce the likelihood of removing an incomplete structure) and stained with alizarin to improve definition of individual rakers. Black and white radiographs of the LHS of specimens (or on their backs if laterally bent) were produced on AGFA Structurix D4 FW X-ray film that was exposed for 30 seconds at 25 kV in a Faxitron X-ray machine. Vertebrae number was then determined by counting vertebrae on enlarged digital images that were created using a Microtec ScanMaker i800 scanner. Following Raadik (2014), counts were taken from the first vertebral centrum adjacent to the occipital condyle of the cranium and excluded the hypural vertebra. The number of neural spines assisted counts where compressed vertebrae were observed.

Dorsal, anal, pectoral and pelvic fin segmented ray counts were separated into branched and unbranched categories, lower (including the raker in the angle of the arch) and upper limb gill raker counts were recorded separately, and the setback of the origin of the dorsal fin relative to the origin of the anal fin was also measured (see Raadik 2014). Unsegmented procurrent fin rays were excluded from fin ray counts.

In addition to TL and SL, 29 morphometric characters were measured in this study (Fig. 1). The definition of these characters follows Raadik (2014) except for GD (gape depth)—the distance between the tip of the lower jaw to the equidistant point between the posterior margins of the lower jaw, measured from ventral surface; KD (keel depth)—the maximum height of the keel; KL (keel length)—the length between the anterior and posterior extent of the keel, measured from the ventral surface; CL (caudal fin length)—standard length subtracted from total length and LCP (caudal peduncle length)—from the hypural crease, through the midline of the body, to the point above

the posterior margin of anal fin base. The following 14 meristic characters were also enumerated: dorsal fin rays—branched (DRB), dorsal fin rays—total segmented (DRS), longest dorsal fin ray (LDR), anal fin rays—branched (ARB), anal fin rays—total segmented (ARS), longest anal fin ray (LAR), caudal fin rays—branched (CRB), caudal fin rays—total segmented (CRS), pectoral fin rays (PecR), pelvic fin rays (PelR), gill rakers lower limb (GRL), gill rakers upper limb (GRU), gill rakers total (GRT) and total number of vertebrae (Ver).

Due to the diminutive size of *Galaxiella pusilla* s.l. and the possibility of measurement error influencing analysis outcomes, repeatability estimates for morphometric measurements and meristic counts were conducted three times on five female and five male specimens from the Crawford River population (see below for repeatability analysis method).

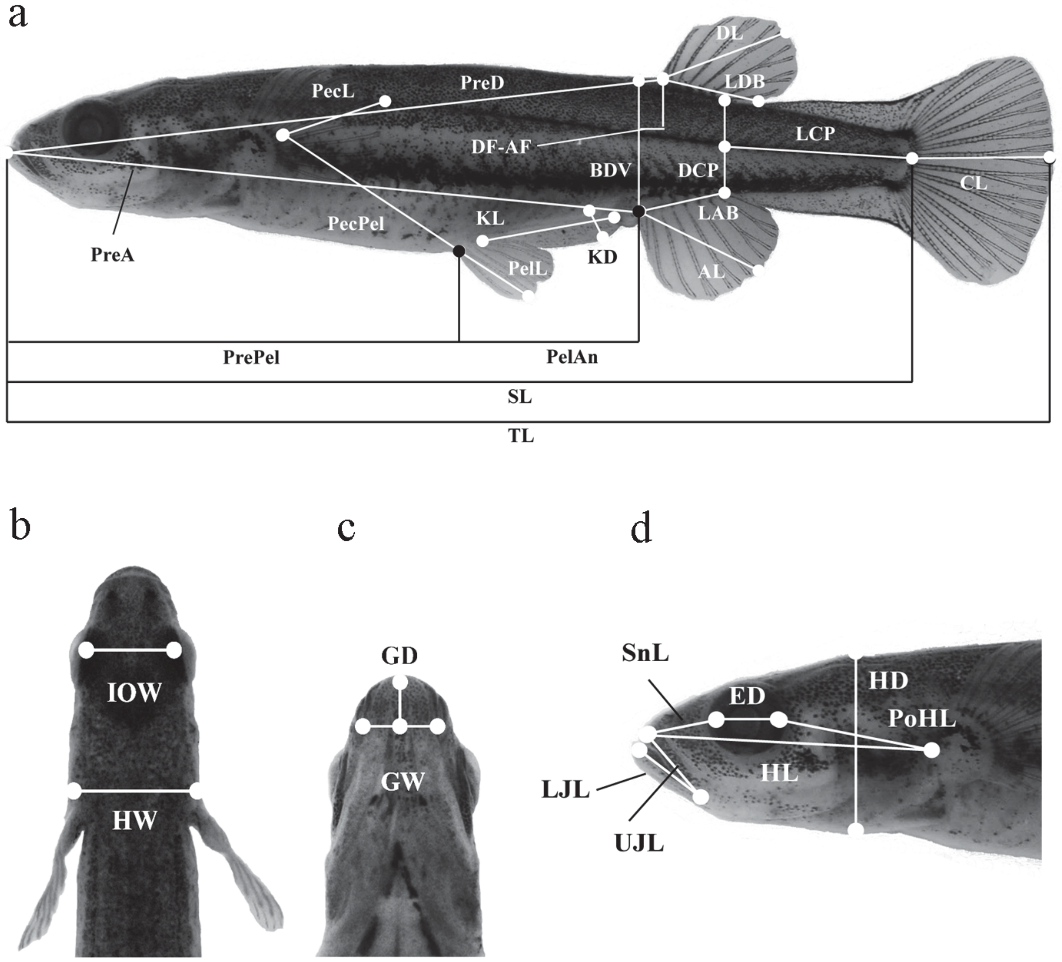


FIGURE 1. Measurements: a) lateral body and fin; b) dorsal head; c) ventral head; and d) lateral head measurements. AL—anal fin length; BDV—body depth at vent; DCP—caudal peduncle depth; DF–AF—dorsal fin to anal fin setback; DL—dorsal fin length; KD—keel depth; KL—keel length; LAB—anal fin base length; CL—caudal fin length; LCP—caudal peduncle length; LDB—dorsal fin base length; PecL—pectoral fin length; PelL—pelvic fin length; PelAn—distance between pelvic and anal fin bases; PecPel—distance between pectoral and pelvic fin bases; PreA—pre-anal fin length; PreD—pre-dorsal fin length; PrePel—pre-pelvic fin length; SL—standard length; TL—total length. ED—eye diameter; GD—gape depth; GW—gape width; HD—head depth; HL—head length; HW—head width; IOW—inter-orbital width; LJL—lower jaw length; PoHL—post-orbital head length; SnL—snout length; UJL—upper jaw length.

Morphometric and meristic analyses. Prior to any analyses, scatterplots and box plots were generated to test for outliers. None were identified and all data were used in analyses. To take into account differences in quantitative characters due to sexual dimorphism, analyses comparing differences between eastern and western regions were undertaken for males and females separately. However, for meristic characters no significant difference between sexes were found (based on Mann-Whitney U tests after Bonferroni correction), and data for sexes were combined.

In order to compare our results with those of McDowall & Frankenberg (1981), and relate the magnitude of any differences between eastern and western regions of *Galaxiella pusilla* s.l. to those described for the Western Australian conspecifics *G. nigrostriata* and *G. munda*, various indices of morphometric and meristic characters, including ratios, were computed. In addition, given potential issues surrounding the use of ratios in taxonomic studies (Atchley *et al.* 1976), an alternative approach was also followed that involved adjusting morphometric measurements for body size (*sensu* Raadik 2011; Adams *et al.* 2014). This comprised $\log_{10}$ transformation of size and trait measurements before correcting for size, using the regression coefficients (β) derived from a linear regression: $y_i = \log_{10}(\chi_i)—\beta \log_{10}(SL_i)$, where y_i and χ_i are the adjusted and raw values for the character in individual *i* and SL_i is the standard length of individual *i*. Where characters were found to be highly correlated after size correction (i.e. r >0.7), redundant characters were removed from further analyses to facilitate multivariate comparisons (see Results). Similarly, for meristic characters, Spearman rank correlations were computed to test for co-correlated characters and redundant characters removed where correlations were high (r_s >0.7) (see Results). Spearman rank correlations also indicated that none of the retained meristic characters were highly correlated with size (r_s < 0.5).

One-way analyses of variance (ANOVA) tested if quantitative characters (corrected for SL) differed significantly between eastern and western populations. ANOVA was also used to estimate measurement repeatability ('*r*', ranges from 0 to 1, and represents the proportion of variation due to differences among individuals, not due to differences within an individual) calculated as $r = S^2A/(S^2 + S^2A)$, where S^2A is the between group variance ((mean squares between individuals—mean squares for repeat measurements within individuals)/ number of repeat measurements) and S^2 is the within group variance (mean squares for repeat measurements within individuals).

For meristic data, east-west comparisons were undertaken with Mann Whitney tests. To investigate separation of the regions based on the traits, a discriminant function analysis (DFA) was undertaken on quantitative (sexes analysed separately) and meristic data. ANOVA was conducted using R version 2.8.1 (R Development Core Team 2009), while Mann Whitney tests, DFA, Pearson correlations and Spearman correlations were conducted with SYSTAT version 13 (Systat Software Inc, San Jose, USA). For individual traits, the Bonferroni correction was applied when identifying significant differences at the 5% level, which equated to an experiment-wide error rate of $\alpha = 0.003$ (i.e. 0.05/14) for meristic characters and $\alpha = 0.002$ (i.e. 0.05/29) for morphometric characters.

Landmark analyses. Landmarks are increasingly being used to characterize variation in body shape within and between fish populations (e.g. Cavalcanti *et al.* 1999; Maderbacher *et al.* 2008; Chakrabarty *et al.* 2010; Ponton *et al.* 2013). In the present study, the LHS of all fish specimens was photographed using a mounted Nikon D70 digital SLR camera equipped with a 105mm macro lens with enhanced lighting against a white background. A ruler with 1 mm increments was included in each image for scale. Each specimen was positioned and photographed twice so that estimates of repeatability could be made. The (*X*, *Y*) coordinates of 12 landmarks on each image were digitised with TPSDIG version 2.17 (Rohlf 2013a) (Fig. 2).

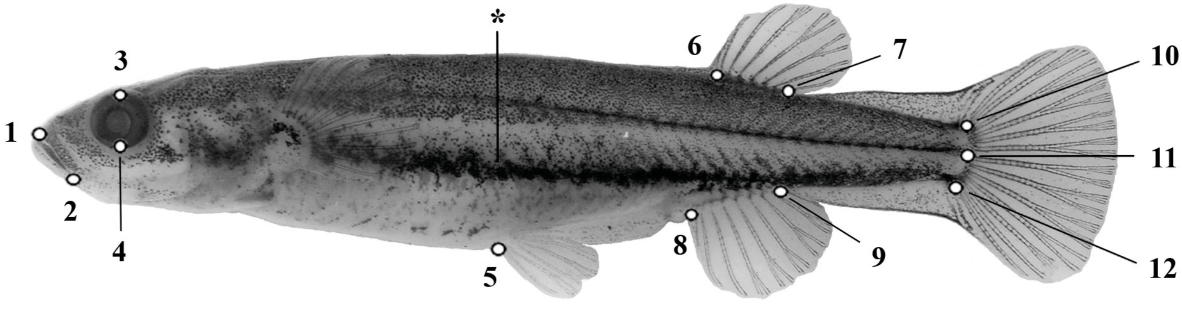


FIGURE 2. Positions of 12 landmarks: 1. tip of snout, 2. posterior extent of lower jaw, 3. superior margin of eye, 4. inferior margin of eye, 5. origin of pelvic fin, 6. origin of dorsal fin, 7. posterior insertion of dorsal fin, 8. origin of anal fin, 9. posterior insertion of anal fin, 10. superior insertion of caudal fin, 11. midpoint of caudal fin base, 12. inferior insertion of the caudal fin, *. inferior margin of lower black band directly above '5' used with '1' and '11' to unbend specimens.

The 12 landmarks were selected to complement key morphometric characters (e.g. position of fins, length of fin bases, length and width of caudal peduncle, length of jaws, eye diameter), whilst representing precise locations to place points, such as the anterior and posterior insertion of fins, maximal extent of snout, jaws and eye. To adjust for the degree of curvature in fish specimens that can develop during preservation, the 'unbend' function in

TPSUTIL version 1.58 (Rohlf 2013b) was applied to all individuals (that fits a quadratic curve as a horizontal straight line, in this case, straightening was based on landmarks '1', '*' and '11'). Shape information was extracted by Procrustes superimposition (to standardise for specimen size, position and orientation) with MORPHOJ version 1.05f (Klingenberg 2011). To determine the ability of body shape to discriminate between eastern and western regions based on Procrustes coordinates, covariance matrix calculations, principal component analysis (PCA), discriminant function analysis (DFA) and Procrustes ANOVA were performed with MORPHOJ. While PCA provides a visual representation of differences without specifying groups, DFA tests the reliability of discrimination between groups using the *T*-square statistic with significance assessed through a permutation test with 10,000 runs. Procrustes ANOVA was used to estimate landmarking error based on Goodall's *F* statistic (*F*) and the associated *P*-value was derived through comparing variation associated with coordinates generated from duplicate images.

Environmental data. Between December 2007–November 2008 and May 2009–May 2010, habitat assessments at a total of 75 sites (35 in the east region and 40 in the west region) were undertaken, and the total length, weight and sex of each *Galaxiella* individual was recorded. Total length measurements in the field (1,850 individuals), were also included in ANOVA analyses to test for morphologic differences between eastern and western populations. These sites were targeted based on historical records of *Galaxiella pusilla* presence, most notably those listed in Saddlier *et al*. (2010). Habitat assessments included estimates of maximum water depth, velocity ('no flow', 'slow', 'moderate', 'fast'), vegetation composition and density, water surface shading, substratum composition and water quality using a WTW Multi 350i multi-parameter meter fitted with ConOx (electrical conductivity, dissolved oxygen and water temperature) and SenTix (pH) probes, and a Merck Turbiquant 1100 turbidity meter. From a broader hydrologic perspective, whether these sites were perennial or intermittently dry is also likely to be important, but could not be reliably assessed for all sites based on short term surveys. Seventeen sites in the east region and 11 sites in the west region were assessed during both the 2007–2008 surveys and 2009–2010 surveys and data included in habitat summaries for both surveys at those sites. Site location coordinates and elevation (m) were also recorded using a Garmin GPS 45 unit.

Results

Morphometric and meristic differences. Meristic and morphometric data from eastern and western regions are summarized in Tables 2–3 and Figs. 3–4. For comparison, data for *Galaxiella nigrostriata* and *G. munda* from McDowall & Frankenberg (1981) are also presented in Tables 4–5. Repeatability assessments indicated a high level of repeatability for most morphometric characters i.e. >0.800. The exceptions were SnL (0.790), LJL (0.736), PecPel (0.714), LAB (0.681) and HL (0.681); they were therefore removed from further analyses (but used in species descriptions).

Total length data collected in the field at various times of the year between 2007–2010 indicated that adult fish from the western region are significantly smaller than those from the eastern region (ANOVA, females $F = 151$, df = 1, P-value <0.001; males $F = 201.7$, df = 1, P-value <0.001; Fig. 5). Pairwise comparisons between size-adjusted quantitative data indicated that characters HD and HW ($r = 0.78$ in females), and HD and GW ($r = 0.72$ in males and 0.75 in females) were highly correlated. Accordingly HD was removed from further analyses, as were GRL and GRU, which were highly correlated with GRT (GRL $r_s = 0.88$ in eastern populations and 0.78 in western populations, GRU $r_s = 0.74$ in eastern populations).

When females were analysed separately, there were significant differences (*P*-values all <0.001) between eastern and western regions for the following morphometric characters after size adjustment: GW, DF-AF, LCP, HW, PrePel, UJL, PreA, PelL, KD, PoHL, and GD (Table S1). When males were analysed separately, there were significant differences (*P*-values all <0.001) between eastern and western *G. pusilla* regions for LCP, GW, HW, PrePel, DF-AF, PreA, PoHL, UJL, KD and BDV. DFA with morphometric characters classified 96% of individual females correctly to regions ($P = <0.001$) and 92% of males ($P = <0.001$). For morphometric characters the regions were separated most strongly by LCP, GW, IOW and KD (both sexes), as well as DF-AF (females) and AL (males) based on forward stepwise selection (Fig. 6).

With sexes combined, significant differences between eastern and western regions were found for the meristic characters LAR, PecR and Ver (Mann Whitney *P*-values of 0.002, 0.001 and <0.001, respectively). DFA with meristic data (sexes combined) classified 93% of fish correctly ($P = <0.001$). The meristic character Ver most strongly separated the regions, along with ARS, ARB, LAR and GRT based on forward stepwise selection. In addition, when combined, morphometric and meristic characters were found to reliably discriminate eastern and western regions.

TABLE 1. Study sites, specimen details and number of individuals used in morphometric measurements and meristic counts. Abbreviations: AMS—Australian Museum, Sydney; NMV—Museum Victoria, Melbourne; SAMA—South Australian Museum, Adelaide. SA = South Australia, SEV = South East Coast (Victoria), Tas = Tasmania, Vic = Victoria.

Site	Region	State	Drainage Division	River Basin	Waterbody	Latitude (S)	Longitude (E)	Collection date	*n* Morpho-metric	*n* Meristic	Museum registration code
1	West	Vic	SEV	Portland	Bridgewater Lakes	38° 19' 13"	141° 24' 16"	2/08/2013	20	20	NMV A31211-001, AMS I.46500-001 SAMA F. 14533,14534
2	West	Vic	SEV	Portland	Fitzroy River	38° 4' 33"	141° 25' 44"	2/08/2013	1	1	NMV A31212-001
3	West	Vic	SEV	Portland	Surrey River	38° 8' 48"	141° 24' 55"	2/08/2013	20	20	NMV A31214-001,002,003
4	West	Vic	SEV	Glenelg	Crawford River	37° 52' 13"	141° 43' 45"	27/06/2013	20	20	NMV A31213-001
5	West	Vic	SEV	Portland	Darlot Creek Tributary	38° 9' 29"	141° 46' 24"	27/06/2013	20	20	NMV A31215-001
6	West	Vic	SEV	Portland	Moyne River Tributary	38° 12' 48"	142° 14' 24"	28/06/2013	20	20	NMV A31216-001
7	West	Vic	SEV	Barwon	Gosling Creek	38° 26' 17"	143° 49' 3"	28/06/2013	2	2	NMV A31217-001
8	West	Vic	SEV	Barwon	Barwon River East Branch	38° 28' 11"	143° 44' 38"	17/07/2013	20	20	NMV A31218-001
9	West	SA	SEV	Millicent	Hammerhead Lagoon	38° 3' 1"	140° 57' 15"	08/09/2008	0	12	NMV A31219-001
10	West	SA	SEV	Millicent	Picks Swamp	38° 2' 33"	140° 54' 43"	12/04/2010	0	6	NMV A31220-001
11	West	SA	SEV	Millicent	Reedy Creek Wilmont Drain	37° 12' 2"	140° 10' 20"	14/04/2010	0	2	NMV A31221-001
12	West	SA	SEV	Millicent	Death Hole Outlet Drain	37° 27' 50"	140° 5' 55"	15/04/2010	0	3	NMV A31222-001
13	West	Vic	SEV	Glenelg	Big Swamp (Grampians)	37° 18' 16"	142° 11' 31"	01/05/1996	0	20	NMV A30675-001
14	East	Vic	SEV	Bunyip	Dandenong Creek Tributary	37° 57' 37"	145° 14' 2"	5/07/2013	20	20	NMV A31223-001
15	East	Vic	SEV	Bunyip	Tuerong Creek	38° 16' 23"	145° 4' 16"	5/07/2013	20	20	NMV A31224-001
16	East	Vic	SEV	Bunyip	King Parrot Creek	38° 10' 27"	145° 52' 7"	5/07/2013	20	20	NMV A31225-001
17	East	Vic	SEV	Latrobe	Moe Main Drain	38° 10' 22"	146° 14' 52"	29/07/2013	20	20	NMV A31226-001
18	East	Vic	SEV	Latrobe	Morwell River	38° 13' 40"	146° 21' 39"	29/07/2013	23	23	NMV A31227-001
19	East	Vic	SEV	Sth Gippsland	Merriman Creek	38° 14' 54"	146° 49' 26"	19/07/2013	20	20	NMV A31228-001
20	East	Vic	SEV	Thomson	Perry River	37° 55' 17"	147° 16' 31"	26/07/2013	20	20	NMV A31229-001
21	East	Vic	SEV	Mitchell	Cobblers Creek	37° 51' 17"	147° 36' 19"	26/07/2013	20	20	NMV A31230-001
22	East	Tas	Tas	East coast	Icena Creek	40° 58' 12"	148° 9' 23"	27/05/2008	0	10	NMV A31231-001
23	East	Tas	Tas	Ringarooma	Big Waterhouse Lake	40° 53' 33"	147° 36' 53"	28/05/2008 1/07/2009	0	9	NMV A31232-001
24	East	Vic	SEV	Bunyip	Cardinia Creek (holotype & paratypes)	38° 7' 52"	145° 24' 29"	05/05/1936	0	5	NMV A97, A98, A388, A389, A390

TABLE 2. Distribution of selected counts for specimens examined of *Galaxiella pusilla* s.s. ('east') and *Galaxiella toourtkoourt* ('west').

Character	Holotype / Allotype	Mode	Median	SD	SE	90 % Range	100 % Range	*n*
East (*G. pusilla* s.s.)								
Dorsal fin rays—branched	4	4	4.0	0.72	0.05	2–4	0–5	177
Dorsal fin rays—total segmented	6	7	7.0	0.54	0.04	6–7	6–8	182
Longest dorsal ray		4	4.0	0.60	0.04	3–5	2–5	180
Anal fin rays—branched		5	5.0	0.92	0.07	3–6	0–7	176
Anal fin rays—total segmented	8	8	8.0	0.66	0.05	7–9	7–10	181
Longest anal ray		4	4.0	0.62	0.05	4–5	3–7	178
Caudal rays—branched	9	9	9.0	1.59	0.15	7–11	1–11	115
Caudal rays—total segmented	13	13	13.0	0.40	0.03	13–14	11–15	179
Pectoral fin rays	13	12	12.0	0.67	0.05	11–13	10–13	182
Pelvic fin rays	5	5	5.0	0.13	0.01	5–5	4–5	182
Gill rakers lower	10	10	11.0	0.85	0.07	9–12	8–13	168
Gill rakers upper		6	6.0	0.61	0.05	5–6	4–7	165
Gill rakers total		16	16.0	1.19	0.09	14–18	13–20	159
Vertebrae	38	39	39.0	0.68	0.05	38–40	36–41	177
West (*G. toourtkoourt*)								
Dorsal fin rays—branched	4 / 3	3	3.0	0.90	0.08	2–5	1–5	130
Dorsal fin rays—total segmented	7 / 6	7	7.0	0.59	0.05	6–7	5–8	146
Longest dorsal ray	4 / 3	4	4.0	0.59	0.05	3–4	2–5	145
Anal fin rays—branched	5 / 5	5	5.0	1.12	0.10	3–6	1–7	129
Anal fin rays—total segmented	8 / 8	8	8.0	0.68	0.06	7–9	7–10	145
Longest anal ray	4 / 4	4	4.0	0.58	0.05	4–5	3–6	141
Caudal rays—branched	10 / 9	9	9.0	1.29	0.12	7–11	2–11	111
Caudal rays—total segmented	13 / 13	13	13.0	0.30	0.02	13–14	12–15	144
Pectoral fin rays	13 / 12	12	12.0	0.70	0.06	11–13	8–13	146
Pelvic fin rays	5 / 5	5	5.0	0.16	0.01	5–5	5–6	146
Gill rakers lower	11 / 10	10	10.0	0.87	0.08	9–12	9–12	122
Gill rakers upper	6 / 6	5	5.5	0.68	0.06	5–6	3–7	116
Gill rakers total	17 / 16	16	16.0	1.14	0.11	14–18	13–19	111
Vertebrae	36 / 37	36	37.0	0.87	0.07	36–38	34–38	140

Eastern and western regions could also be discriminated based on the 12 landmarks and associated shape information, including taking into account measurement and placement error (females: Goodall's $F = 46.82$, $P = <0.001$ and males: Goodall's $F = 39.92$, $P = <0.001$). In the PCA results, separation between individuals was pronounced on PC2 for both female and male specimens (Fig. 7). Distinguishing shape features associated with PC2 included a more slender profile, more pronounced setback of the dorsal fin origin to anal fin and shorter pre-pelvic length for the eastern region compared to the western region. A DFA indicated a significant difference between eastern and western populations based on the Procrustes coordinates (females: T-square = 271.374, $P = <0.001$; and males: T-square = 229.286, $P = <0.001$) (Fig. 8). Correct classification of eastern and western females was high at 89.3% and 88.5 %, respectively. Similarly, correct classification of eastern and western males was 91.0 and 90.3 %, respectively.

Habitat assessments. The habitat characteristics of sites where fish in eastern and western regions were found are summarised in Appendix 2. Habitat can be described as low-moderate elevation (range and mean for eastern sites was 8–111 m and 54 m, and for western sites 7–376 m and 104 m), shallow (maximum water depth range and

TABLE 3. Morphometric data summary for specimens examined of *Galaxiella pusilla* s.s. ('east') and *Galaxiella toourtkoourt* ('west') where sexes are separated. Specific measurements for *Galaxiella toourtkoourt* holotype and allotype are also provided. Excluding total length and standard length, figures are given as percentages of the first character over the second character in the first column.

	East Female (*G. pusilla* s.s.)				East Male (*G. pusilla* s.s.)				West Female (*G. toourtkoourt*)					West Male (*G. toourtkoourt*)				
	min	max	mean	SD	min	max	mean	SD	Holotype	min	max	mean	SD	Allotype	min	max	mean	SD
Standard length	21.4	32.7	27.2	2.86	18.1	28.2	23.6	2.20	29.2	20.6	30.6	24.2	2.3	20.7	16.7	24.0	20.2	1.48
Total length	23.4	38.1	31.5	3.28	20.0	33.5	27.5	2.53	33.8	23.7	34.6	28.3	2.6	24.5	19.4	27.8	23.7	1.77
Body depth at vent/standard length	12.0	15.5	13.8	0.80	11.8	15.4	13.6	0.77	15.2	10.3	16.8	14.2	1.0	13.5	12.8	15.8	14.1	0.78
Length of caudal peduncle/standard	18.4	25.5	22.2	1.36	20.3	26.0	22.9	1.36	20.4	17.2	23.8	20.5	1.4	22.7	17.9	24.4	21.1	1.49
Predorsal length/standard length	69.0	80.8	75.2	1.71	66.0	77.8	73.9	1.74	75.2	70.5	85.7	75.6	2.2	76.2	71.3	78.1	74.5	1.59
Length of dorsal fin base/standard	6.5	10.2	8.3	0.83	6.8	10.6	8.7	0.81	9.1	6.6	10.9	8.6	0.9	8.2	7.2	11.7	9.0	0.87
Maximum length of dorsal fin/standard	12.8	17.2	14.6	0.92	12.7	17.7	15.1	0.91	16.6	12.6	17.3	15.1	1.0	14.4	12.5	18.8	15.2	1.30
Length of anal fin base/standard length	9.7	13.3	11.0	0.80	10.2	14.5	12.1	0.88	10.7	9.2	14.2	11.4	1.0	10.5	9.4	15.3	12.6	1.25
Maximum length of anal fin/standard	14.1	19.1	15.9	0.87	15.2	19.9	17.2	0.92	16.1	12.6	20.0	16.4	1.3	15.8	13.8	19.6	16.9	1.27
Maximum length of pectoral	9.6	14.0	11.8	0.83	10.7	15.2	12.5	0.84	11.9	9.8	15.1	12.5	1.0	13.5	9.3	15.9	12.9	1.39
Maximum length of pelvic fin/standard	8.0	11.3	9.9	0.69	8.7	12.2	10.4	0.67	11.9	9.3	12.4	10.7	0.7	11.5	8.9	13.0	11.0	0.83
Maximum length of caudal	9.3	20.2	16.1	1.67	10.5	20.2	16.8	1.68	15.9	12.3	27.3	17.2	2.4	18.3	13.7	20.9	17.2	1.54
Prepelvic length/standard length	46.6	54.2	50.5	1.73	45.9	53.4	49.7	1.43	52.7	47.7	57.4	52.1	1.6	52.4	46.8	55.3	51.4	1.77
Pectoral-pelvic length/standard length	22.2	31.1	25.7	1.90	19.4	27.2	23.7	1.61	24.6	22.0	37.4	25.8	2.3	21.0	19.9	27.5	23.2	1.84
Pelvic-anal length/standard length	17.9	24.0	20.6	1.34	16.9	22.8	18.8	1.19	20.8	18.6	26.1	21.2	1.5	20.0	17.3	22.4	19.5	1.23
Head length/standard length	19.7	26.8	23.0	1.37	19.7	26.9	23.8	1.25	27.8	20.6	28.4	24.6	1.5	28.3	22.4	28.3	25.4	1.27
Dorsal-anal fin setback/standard length	0.0	3.9	2.2	0.88	0.5	5.7	3.1	1.02	0.4	-2.5	2.8	0.6	1.1	1.5	-2.6	3.9	2.0	1.03
Keel length/standard length	12.1	19.1	15.1	1.39	11.2	17.6	14.5	1.30	16.8	10.5	19.6	15.4	1.4	15.4	11.3	17.9	15.1	1.37
Keel depth/standard length	1.3	2.9	2.0	0.37	0.8	3.0	1.9	0.41	2.8	1.6	3.4	2.5	0.4	2.6	1.6	3.3	2.3	0.36
Head width/head length	61.4	87.5	70.4	5.38	56.6	81.9	65.2	4.97	62.2	60.2	87.8	72.7	5.6	56.8	40.3	80.4	66.6	6.90
Head depth/head length	55.9	83.1	68.1	4.64	60.1	77.7	66.8	3.59	61.3	60.2	90.5	70.0	5.4	60.2	58.6	84.7	68.3	5.24
Snout length/head length	13.6	26.9	20.9	2.05	15.9	24.6	20.7	1.88	21.0	13.9	25.4	21.0	1.9	20.9	16.0	24.3	20.3	1.99
Post-orbital head length/head length	42.9	62.6	50.5	3.21	43.0	53.5	48.9	2.52	53.8	39.5	59.0	49.9	3.9	54.5	42.2	57.6	48.9	3.73
Interorbital width/head length	37.2	58.9	44.8	4.44	36.7	59.3	43.4	4.79	38.6	35.5	52.0	42.5	3.4	37.1	30.0	52.6	40.2	4.20
Diameter of eye/head length	25.3	36.4	30.6	2.10	28.1	35.9	32.0	1.72	24.4	24.4	36.4	30.2	3.0	30.1	29.3	37.2	33.0	1.93
Length of upper jaw/head length	18.5	31.2	24.2	2.21	18.3	28.6	23.7	2.00	27.7	20.2	28.8	25.3	2.0	26.7	15.2	29.3	24.1	2.58
Length of lower jaw/head length	16.6	26.9	22.4	2.21	15.9	27.4	22.1	2.12	26.9	18.8	27.3	23.5	2.2	25.5	14.0	26.7	22.0	2.52
Width of gape/head length	21.3	38.9	27.8	3.94	21.1	34.2	25.6	2.62	26.9	23.3	38.4	29.6	3.1	23.2	20.8	33.6	26.3	2.96
Depth of gape/head length	14.9	27.1	22.4	2.16	14.0	26.1	21.6	1.98	22.7	17.4	30.5	22.5	2.7	24.3	14.6	27.9	21.0	2.77
Depth of caudal peduncle/length of	34.3	53.6	43.8	3.30	36.9	52.9	43.2	3.72	54.9	38.5	60.1	48.5	5.6	43.3	36.1	59.4	47.2	5.10
Predorsal length/pre anal length	99.1	113.9	107.0	2.57	94.4	125.6	108.4	3.49	102.9	97.6	110.7	104.7	2.7	105.5	100.0	112.5	106.4	2.76
Number of fish measured	84				79					61					62			

mean for both eastern and western sites was 0.1–>2.0 m and 1.1 m), low water velocity (percentage of sites observed to have 'no water flow' or 'slow water flow' at the time of surveys of eastern and western sites was 96.2% and 94.1%, respectively), clay-silt dominated substratum (mean percentage composition of clay and silt for eastern sites was 32.9 and 42.7 and for western sites it was 33.8 and 43.0) and partially shaded (range and mean for eastern sites was 0–80 % and 35.9 %, and for western sites was 0–100 % and 27.0 %).

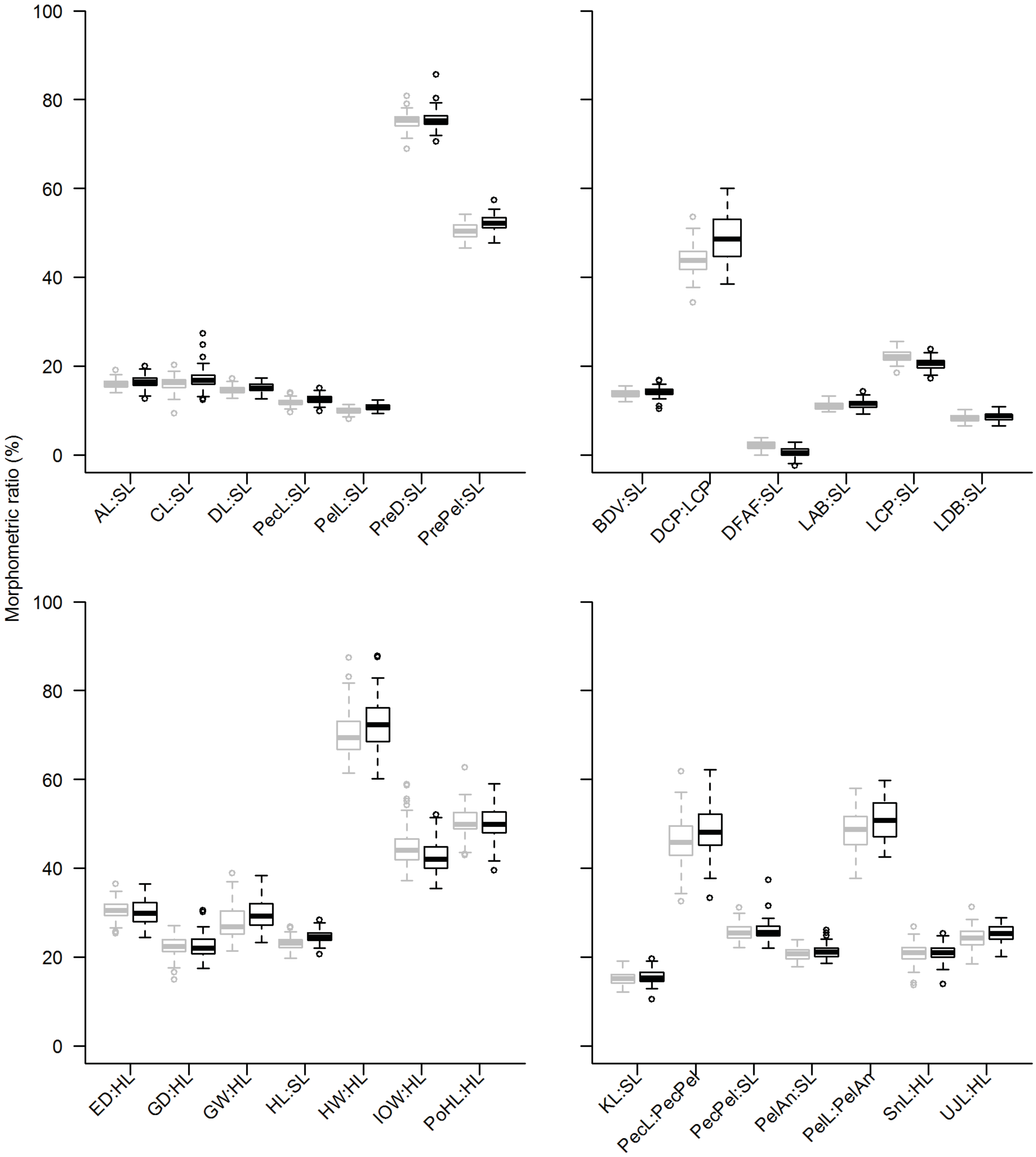


FIGURE 3. Boxplots of morphometric ratio data for female *Galaxiella pusilla* s.s. (black) and *Galaxiella toourtkoourt* (grey). Values are given as percentages of the first measurement over the second measurement listed on the x axes. Outliers that are >1.5 times the interquartile range above the upper quartile or below the lower quartile are indicated by a dot. See Fig. 1 for measurement descriptions.

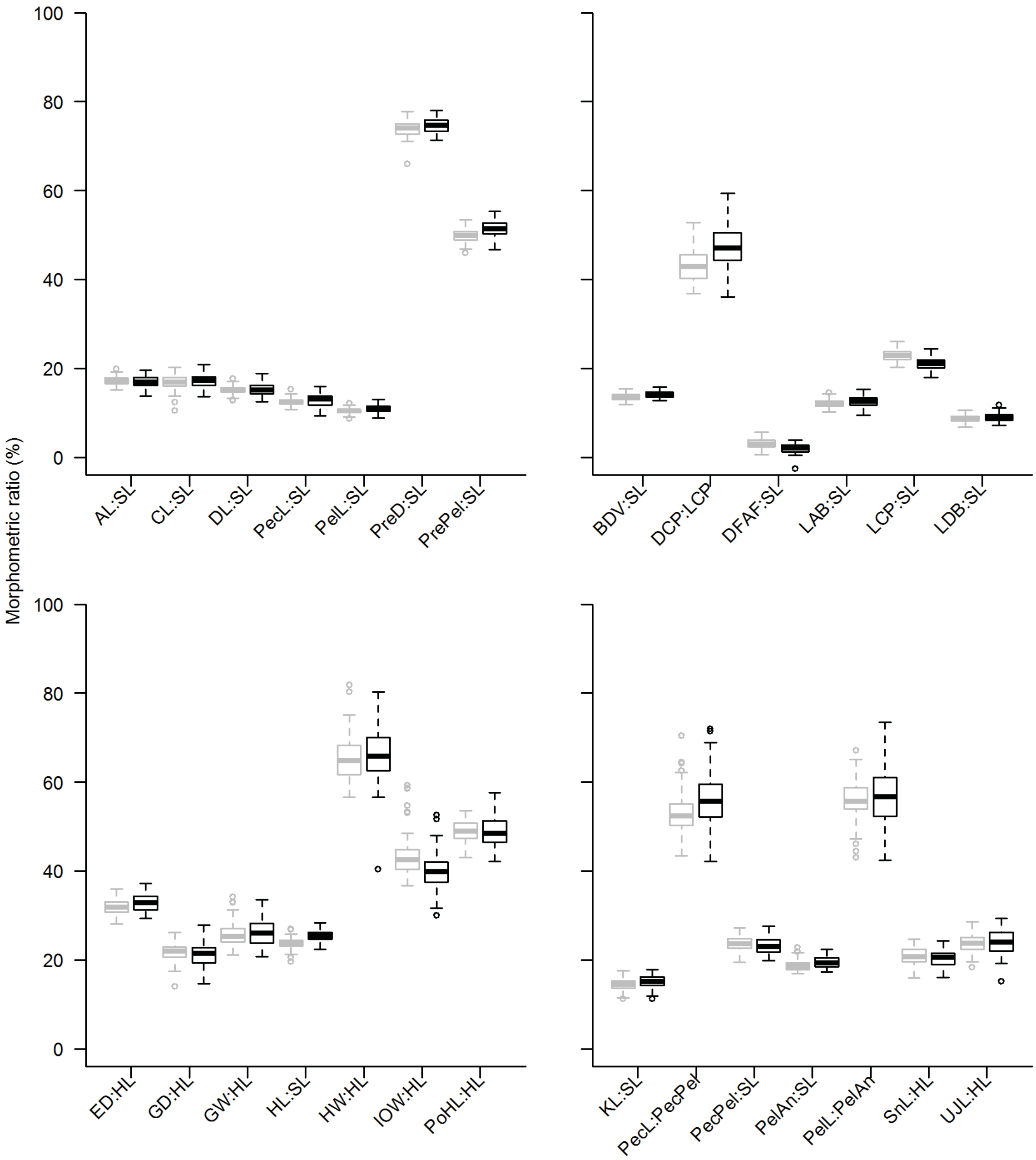


FIGURE 4. Boxplots of morphometric ratio data for male *Galaxiella pusilla* s.s. (black) and *Galaxiella toourtkoourt* (grey). Values are given as percentages of the first measurement over the second measurement listed on the x axes. Outliers that are >1.5 times the interquartile range above the upper quartile or below the lower quartile are indicated by a dot. See Fig. 1 for measurement descriptions.

The vegetation community at sites tended to be dominated by emergent aquatic vegetation (mean percentage cover for eastern and western sites was 45.0 % and 35.8 %, respectively) particularly species of *Juncus*, *Persecaria, Phragmites*, *Triglochin* and *Typha*. Although the cover of submerged aquatic macrophytes tended to be less than the cover of emergent aquatic macrophytes (i.e. mean percentage cover for eastern and western sites was 12.3 % and 17.2 %, respectively), at some sites species of *Myriophyllum* and *Potamogeton* as well as charophytes were dominant. *Melaleuca* trees were also a dominant feature of the vegetation community at some sites (at 16 eastern and five western sites, they comprised 10% or more of the total aquatic and terrestrial vegetation cover), but in most cases emergent vegetation throughout the water body provided the majority of water surface shading. The cover of coarse particulate organic matter (CPOM) at each site tended to be high (mean percentage cover for eastern and western sites was 30.6 % and 25.0 %, respectively), however, large wood debris cover (pieces of wood longer than 1 m and a diameter > 0.1 m) was not a dominant feature at most sites (mean percentage cover for eastern and western sites was 3.6 % and 1.9 %, respectively).

TABLE 4. Comparison of selected counts for specimens examined of *Galaxiella pusilla* s.s. ('east') and and *Galaxiella toourtkoourt* ('west') with McDowall and Frankenberg (1981) for *Galaxiella nigrostriata* and *Galaxiella munda.*

		East (*G. pusilla ss*)	West (*G. toourtkoourt*)	*G. nigrostriata*	*G. munda*
Dorsal fin rays	5		1		
- segmented	6	77	45	10	8
	7	84	72	10	24
	8	2	5		5
	n	163	123	20	37
Anal rays	7	21	16		
- segmented	8	105	69	2	
	9	33	35	11	1
	10	4	3	3	12
	11			3	19
	12				5
	n	163	123	19	37
Caudal rays	10				
	11	1			
	12	8	2	1	
	13	141	111	4	1
	14	12	7	14	32
	15	1	1	1	2
	n	163	121	20	35
Pectoral rays	9				1
	10	6			15
	11	54	20	3	9
	12	91	79	14	3
	13	12	23	4	
	14			1	
	n	163	122	22	28
Pelvic rays	3				
	4	3			
	5	160	119	19	2
	6		4		33
	7				2
	n	163	123	19	37
Gill rakers	12				
- total	13	2	1		
	14	7	6	1	
	15	29	23	4	
	16	48	35	9	5
	17	42	29	2	4
	18	12	8	2	3

......continued on the next page

TABLE 4. (Continued)

		East (*G. pusilla ss*)	West (*G. toourtkoourt*)	*G. nigrostriata*	*G. munda*
	19	4	1		3
	20	1			1
	n	146	103	18	16
Vertebrae	34		1		
	35		6		
	36	1	43		
	37	2	41		
	38	31	26	2	3
	39	108		22	7
	40	16		14	15
	41	1		8	18
	42			3	19
	43			2	4
	n	159	117	51	66

Fish from eastern and western regions were found in a broad range of water quality conditions. Water temperatures at the time of surveys (covering conditions during both warmer and cooler months of the year) ranged from 5.8–24.8 ºC (mean 14.8) for eastern sites and from 5.2–26.9 ºC (mean 16.3) for western sites. Measurements of pH from eastern sites ranged from 5.0–7.8 (mean 6.6) and for western sites from 5.3–9.3 (mean 7.6), while dissolved oxygen from eastern sites ranged from 18.2–130 % (mean 56.9) and for western sites from 20.0–263.0 % (mean 81.3). Water clarity was also quite variable, and ranged from 1–133 NTU (mean 26.6) for eastern sites and 1–96 NTU (mean 15.7) for western sites. Of particular note are observed salinity differences between regions, where levels of water electrical conductivity within eastern sites ranged from 36–3,070 µS/cm (mean 708) and within western sites from 94–13,620 µS/cm (mean 2,455).

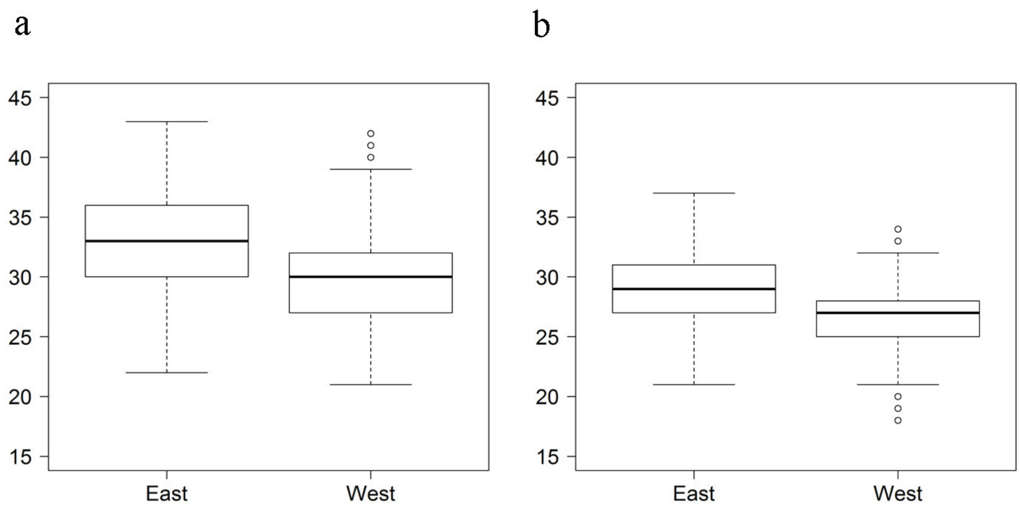


FIGURE 5. Boxplots of total length (mm) measurements in the field of *Galaxiella pusilla* s.s. ('east') and *Galaxiella toourtkoourt* ('west') for a) females (east n = 451, west n = 478) and b) males (east n = 459, west n = 462). Outliers that are >1.5 times the interquartile range above the upper quartile or below the lower quartile are indicated by a dot.

TABLE 5. Comparison of morphometric data for specimens examined of *Galaxiella pusilla* s.s. ('east') and *Galaxiella toourtkoourt* ('west') (where sexes are combined) with McDowall and Frankenberg (1981) for *Galaxiella nigrostriata* and *Galaxiella munda*. Figures are given as percentages of the first character over the second character in the first column.

	East (*G. pusilla* s.s.)				West (*G. toourtkoourt*)				*G. nigrostriata*				*G. munda*			
	min	max	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	mean	SD
Standard length/total length	83.2	91.5	85.9	1.28	78.5	89.0	85.3	1.44	83.7	90.3	85.9	1.75	82.2	87.7	84.9	1.29
Body depth at vent/standard length	11.8	15.5	13.7	0.79	10.3	16.8	14.2	0.94	13.5	18.5	16.5	1.21	10.5	14.3	12.8	1.12
Length of caudal peduncle/standard length	18.4	26.0	22.5	1.40	17.2	24.4	20.8	1.48	18.7	23.5	21.0	1.51	15.2	22.5	18.9	1.73
Predorsal length/standard length	66.0	80.8	74.5	1.84	70.5	85.7	75.1	2.00	70.5	75.0	72.6	1.04	70.2	76.7	73.3	1.41
Length of dorsal fin base/standard length	6.5	10.6	8.5	0.85	6.6	11.7	8.8	0.92	7.1	8.8	7.9	0.61	6.7	9.8	8.3	0.73
Length of anal fin base/standard length	9.6	14.5	11.6	0.99	9.2	15.2	12.0	1.28	11.1	13.8	12.6	0.88	12.3	16.4	14.6	1.07
Prepelvic length/standard length	45.9	54.2	50.1	1.63	46.8	57.4	51.8	1.76	46.1	52.8	50.6	1.64	40.5	52.6	49.1	3.04
Pectoral-pelvic length/standard length	19.4	31.1	24.8	2.03	19.9	37.4	24.5	2.48	27.4	31.8	29.5	1.19	23.2	33.3	29.4	2.38
Pelvic-anal length/standard length	16.9	24.0	19.7	1.58	17.3	26.1	20.3	1.63	15.0	17.9	16.6	0.90	15.5	19.7	17.6	1.08
Head length/standard length	19.6	26.9	23.4	1.37	20.6	28.4	25.0	1.45	20.9	23.9	22.4	0.82	17.4	22.8	19.9	1.16
Width of gape/head length	21.1	38.8	26.8	3.53	20.8	38.4	27.9	3.45	21.7	27.8	24.4	1.85	20.8	30.0	25.4	2.83
Head width/head length	56.6	87.5	67.9	5.79	40.3	87.8	69.6	7.00	53.8	66.7	59.4	4.32	50.0	62.5	55.8	3.40
Head depth/head length	55.8	83.1	67.5	4.20	58.6	90.5	69.2	5.41	55.5	66.7	60.3	3.15	50.0	66.7	55.9	4.16
Snout length/head length	13.6	26.9	20.8	1.96	13.9	25.4	20.6	2.01	22.2	29.6	26.4	1.93	23.1	35.0	28.2	2.49
Post-orbital head length/head length	42.9	62.6	49.7	2.99	39.5	59.0	49.4	3.86	44.4	54.2	48.4	2.71	41.7	53.6	46.5	2.34
Interorbital width/head length	36.7	59.3	44.1	4.65	30.0	52.6	41.3	4.01	33.3	44.5	37.9	3.36	34.4	45.8	40.8	2.67
Diameter of eye/head length	25.3	36.4	31.2	2.05	24.4	37.2	31.6	2.88	25.0	30.8	28.3	1.57	26.5	35.0	30.4	2.41
Length of upper jaw/head length	18.3	31.2	24.0	2.12	15.2	29.3	24.7	2.40	26.9	33.3	29.1	2.26	29.2	40.0	32.5	2.22
Length of lower jaw/head length	15.9	27.4	22.3	2.16	13.9	27.3	22.8	2.49	25.0	29.6	27.2	1.87	26.7	35.0	30.5	2.18
Depth of caudal peduncle/length of peduncle	34.3	53.6	43.5	3.51	36.1	60.1	47.8	5.40	46.4	61.9	55.8	4.31	40.7	54.5	47.3	4.69
Predorsal length/pre anal length	94.4	125.6	107.7	3.12	97.6	112.5	105.5	2.86	104.7	112.5	107.8	1.98	105.0	111.9	108.3	1.87
Maximum length of dorsal fin/basal length	146.2	218.2	176.1	13.49	124.0	217.4	173.3	14.81	155.6	188.2	175.3	10.33	150.0	200.0	174.5	13.91
Maximum length of anal fin/basal length	116.4	170.3	143.7	9.45	115.0	170.4	139.7	11.48	128.6	166.7	144.1	10.40	125.0	154.5	137.2	7.84
Pectoral fin length/pectoral-pelvic length	32.5	70.4	49.5	6.06	33.2	71.9	52.4	6.93	41.2	51.6	47.8	3.06	39.1	52.9	45.2	3.58
Pelvic fin length/pelvic-anal length	37.8	67.1	52.0	5.82	42.4	73.5	53.8	6.07	50.0	68.8	60.4	5.03	50.0	70.0	60.3	5.66
Number of fish measured	163				123				16				24			

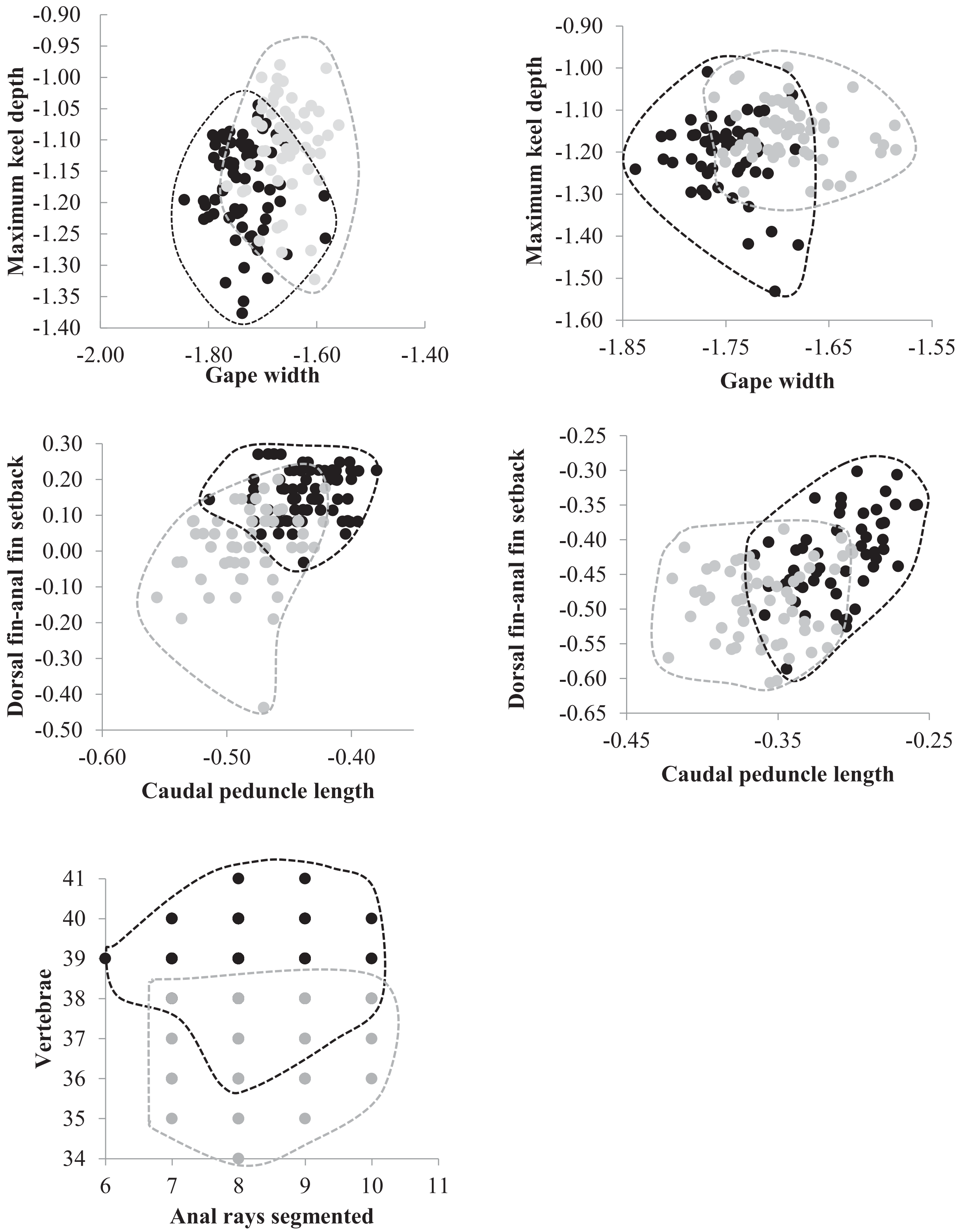


FIGURE 6. Scatterplots for characters found by discriminant function analysis (DFA) to strongly separate *Galaxiella pusilla* s.s. (black) and *Galaxiella toourtkoourt* (grey): a) gape width by maximum keel depth—females, b) caudal peduncle length by dorsal fin-anal fin setback—females, c) gape width by maximum keel depth—males, d) caudal peduncle length by dorsal fin-anal fin setback—males e) anal rays segmented by vertebrae—sexes combined. Envelopes indicate range of values. Morphometric characters are corrected for standard length.

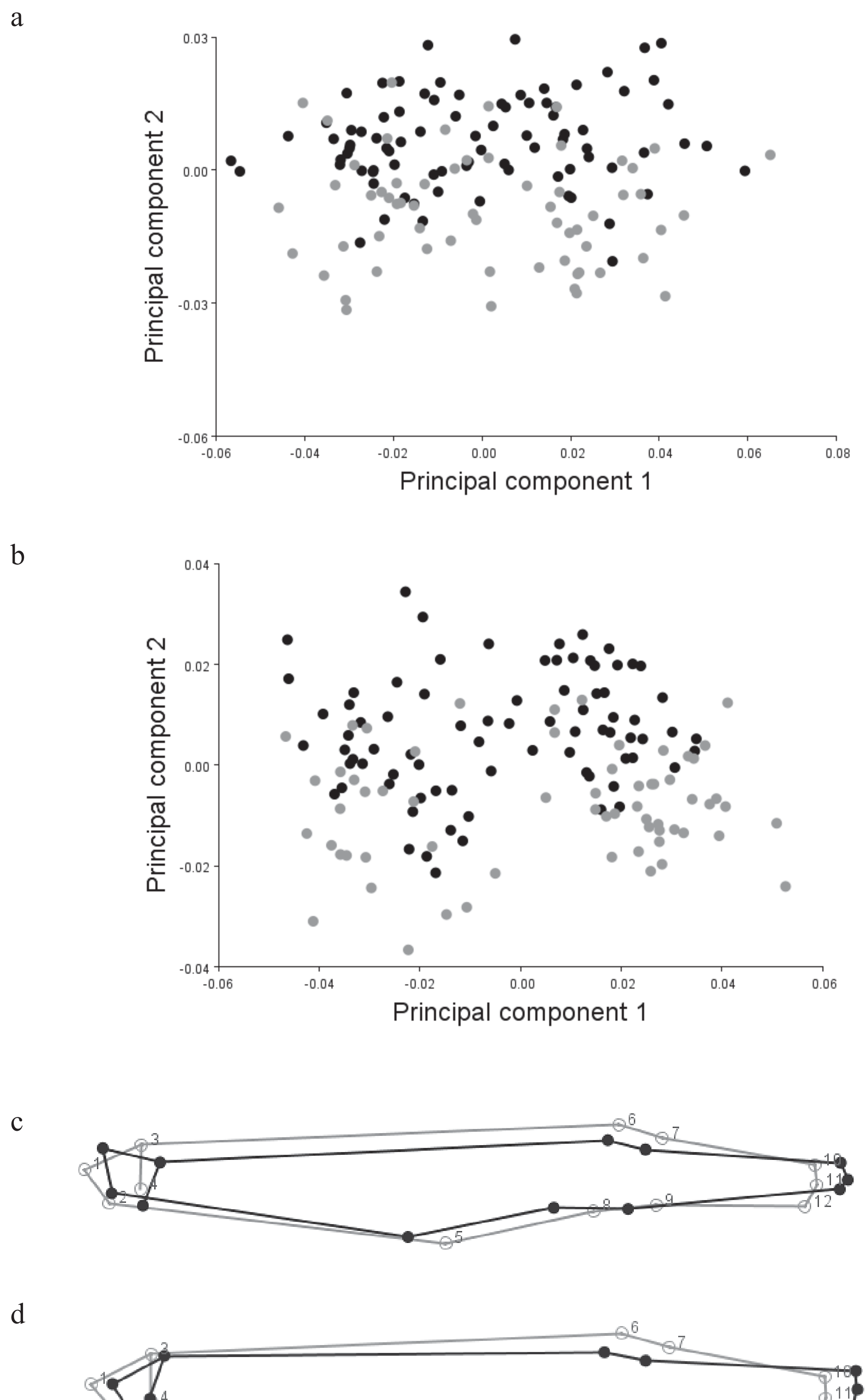
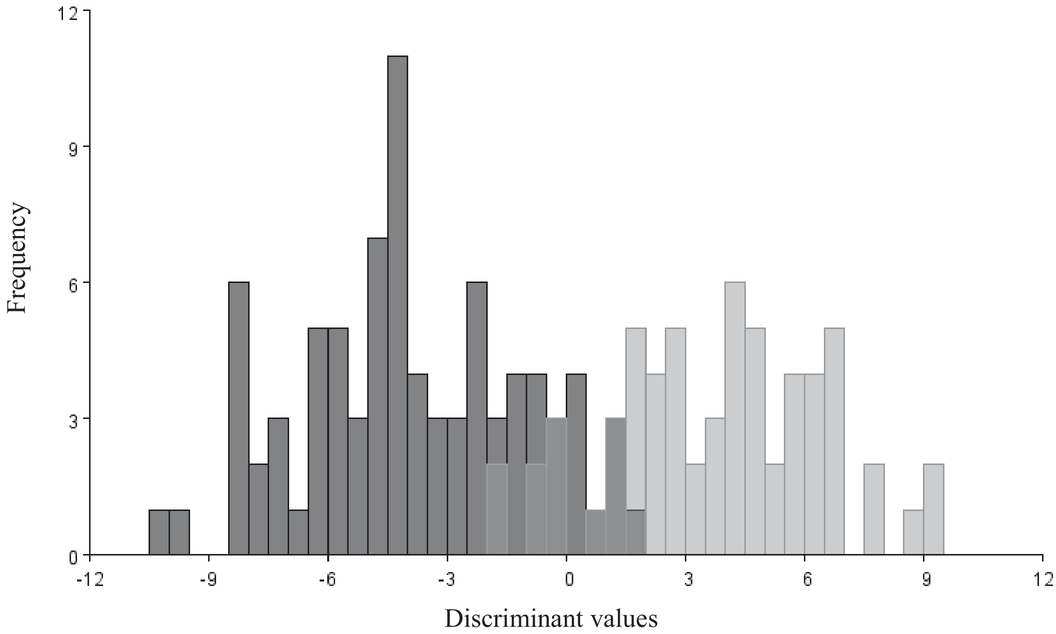


FIGURE 7. Principal components analysis using shape information for *Galaxiella pusilla* s.s. (black) and *Galaxiella toourtkoourt* (grey) generated from 12 landmarks for a) females (PC1 and PC2 explain 52.0% and 14.5% of the total variance, respectively), b) males (PC1 and PC2 explain 54.4% and 14.7% of the total variance, respectively), and shape changes associated with principal component 2 for c) females and d) males.

a



b

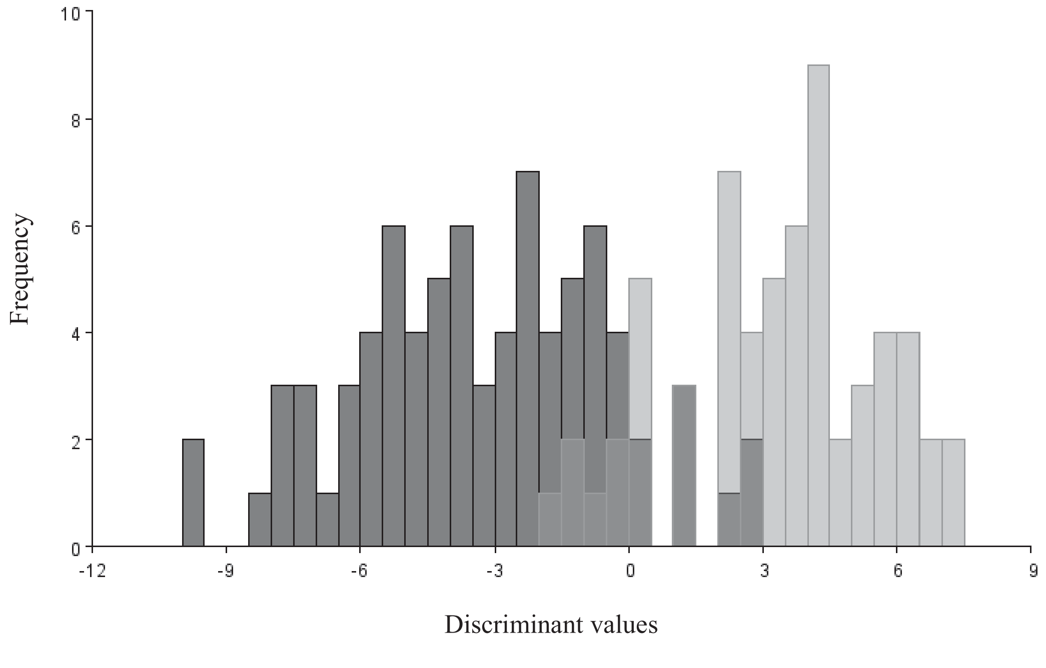


FIGURE 8. Discriminant value histograms using shape information from *Galaxiella pusilla* s.s. (black) and *Galaxiella toourtkoourt* (grey) generated from 12 landmarks for a) females, b) males.

Systematics

Material examined is included under each description listed by state, then by drainage division, river basin within each division, and alphabetically within river basins. Length measurements are provided as SL (standard length) of

individuals examined in each specimen lot. Except for the museum specimens from Museum Victoria, Melbourne (NMV), all new specimens were collected by Rhys A. Coleman (RAC) and James A. Coleman (JAC). Type material for the new species has been lodged in the collection of NMV, Australian Museum, Sydney (AMS), and South Australian Museum, Adelaide (SAMA). For *Galaxiella toourtkoourt*, meristic values in the species description are presented for the holotype followed by the 100% range in brackets for male and female specimens combined. Morphometric values in the species description for *Galaxiella toourtkoourt* females are given as the holotype followed by the 100% range, and given as the allotype followed by the 100% range for male specimens. The *Galaxiella pusilla* s.s. holotype is now substantially degraded, so reliable morphometric measurements (and some meristic counts) were not possible. Accordingly, in the revised species description for *Galaxiella pusilla* s.s., morphometric and meristic values are presented as the mean or mode respectively, followed by the 100% range in brackets. Where meristic characters for the *Galaxiella pusilla* s.s holotype were counted, those values are given after the range.

Genus *Galaxiella* McDowall

Gender: feminine.
Galaxiella McDowall, 1978: 116. Type species: *Galaxias pusillus* Mack, 1936: 101.

A genus of four valid species endemic to Australia, with two native to south-eastern Australia (including Tasmania) and two native to south-western Australia.

Diagnosis (modified from McDowall 1978a and McDowall & Frankenberg 1981). Small, stocky, horizontally-striped galaxiid fishes with dorsal fin origin usually in line with, or posterior to, anal fin origin (occasionally slightly anterior). Pelvic fins present, usually 5–6 rays (range 4–7); pectoral fins inserted high laterally. Caudal fin rounded, usually with 13–14 segmented rays (range 11–15); caudal peduncle flanges very strongly developed, extending forward ventrally to near anal fin base, but with very few procurrent rays. No submandibular laterosensory pores. No median ethmoid ossification. Postcleithrum present. Epipleural and epineural ribs present; no ossified interneurals. Caudal neural and haemal spines slender spikes, not laterally flattened. Vertebrae few, 34–43; branchiostegals few, 3–4. Sexual dimorphism present in two species from south-eastern Australia, including smaller size at maturity, three distinct horizontal black stripes along sides of body and bright orange-red horizontal stripe between lower two black stripes in males.

Key to species of *Galaxiella*

(adapted from McDowall & Frankenberg 1981)

1. Usually 6 pelvic fin rays (range 5–7); usually 10–11 pectoral fin rays (range 9–12); usually 16–19 total gill rakers (range 16–20); usually 40–42 vertebrae (range 38–43). (Western Australia from near Margaret River in the west, Albany to the east and north of Perth) . . . . *Galaxiella munda* McDowall 1978

\- Usually 5 pelvic fin rays (range 4–6); usually 11–13 pectoral fin rays (range 10–14); usually 14–18 total gill rakers (range 12–20) and usually 36–40 vertebrae (range 34–43) 2

2. Usually 14 caudal fin rays (range 12–15); 8 pores in preopercular-suborbital-supramaxillary series; 2 distinct lateral longitudinal dark stripes. (Western Australia from Augusta in the west and Albany to the east and north of Perth) . . . . *Galaxiella nigrostriata* (Shipway 1953)

\- Usually 13 caudal fin rays (range 11–15); 7 pores in preopercular-suborbital-supramaxillary series; 3 distinct lateral longitudinal dark stripes (mid stripe and particularly dorsal stripe can be poorly defined in females). 3

3. Usually 39 vertebrae (range 36–41); origin of dorsal fin distinctly posterior to that of anal fin, with horizontal distance between dorsal fin and anal fin origins 1.4–3.8% of SL (females 1.4–2.8% (range 0.0–3.8), males 2.4–3.8% (range 0.52–5.67)); caudal peduncle of moderate length, 21.3–23.8% of SL (females 21.3–23.1% (range 18.4–25.5), males 21.9–23.8% (range 20.3–26.0)); adult females up to 32.7 mm SL (mean 27.2) and adult males up to 28.2 mm SL (mean 23.6); ventral markings distinct, most commonly a 'v'-shaped dark mark originating in the isthmus and extending as two parallel dotted lines (sometimes discontinuous) to the pelvic fin bases. (South-eastern Australia from Bairnsdale in eastern Victoria to south-east Melbourne, including Flinders Island and north-eastern and north-western Tasmania) . . . . *Galaxiella pusilla* (Mack 1936)

\- Usually 36 vertebrae (range 34–38); origin of dorsal fin is more or less in line with that of anal fin (particularly females), with the horizontal distance between dorsal fin and anal fin origins usually 0.0–2.7% of SL (females 0.0–1.4% (range -2.5–2.8), males 1.3–2.7% (range -2.6–3.9); caudal peduncle relatively short, usually 19.6–21.9% of SL (females 19.6–21.2% (range 17.2–23.8), males 20.1–21.9% (range 17.9–24.4)); adult females up to 30.6 mm SL (mean 24.2) and adult males up to 24.0 mm SL (mean 20.2); ventral markings reduced, most commonly a small number of dark blotches in the vicinity of the isthmus. (South-eastern Australia from Barwon Downs in western Victoria to near the Coorong in south-eastern South Australia) . . . . *Galaxiella toourtkoourt* new species.

Galaxiella toourtkoourt **Coleman & Raadik, new species**
Little galaxias
Tables 2 to 3; Figs. 3 to 4, 9, 10a, 11 and 12

Galaxias pusillus (non Mack, 1936)—Andrews 1976: 324 (*partim*).
Brachygalaxias pusillus (non Mack, 1936)—Scott 1942: 55 (*partim*); Whitley 1956b: 39 (*partim*); 1957: 6; 1960: 29; Scott 1966: 246 (*partim*); Frankenberg 1967: 227 (partim); Lake 1971: 20 (*partim*); Scott 1971: 3 (*partim*); Chessman & Williams 1974: 168; Pollard 1980: 348 (*partim*).
Brachygalaxias pusillus pusillus (non Mack, 1936)—Munro 1957: 18 (*partim*); Frankenberg 1969: 185 (*partim*); 1971: 94 (*partim*).
Galaxiella pusilla (non Mack, 1936)—Backhouse & Vanner 1978: 129 (*partim*); Lake 1978: 26 (*partim*); McDowall 1978a: 116 (*partim*); McDowall 1980: 55 (*partim*); McDowall & Frankenberg 1981: 552 (*partim*); Backhouse 1983: 6 (*partim*); Cadwallader & Backhouse 1983: 75 (*partim*); Humphries 1983: 4 (*partim*); Jackson & Davies 1983: 39; Last *et al.* 1983: 201 (*partim*); Merrick & Schmida 1984: 98 (*partim*); Beck 1985: 12; Allen 1989: 44 (*partim*); Berra & Allen 1989: 293 (*partim*); Paxton *et al.* 1989: 179 (*partim*); Fulton 1990: 39 (*partim*); Koehn & Morison 1990: 20 (*partim*); Andrews 1991: 59 (*partim*); Koehn & Raadik 1991: 77 (*partim*); Pen *et al.* 1993: 848 (*partim*); Humphries 1995: 1159 (*partim*); Unmack & Paras 1995: 398 (*partim*); Westbury 1995: 1 (*partim*); Chilcott & Humphries 1996: 147 (*partim*); McDowall & Fulton 1996: 70 (*partim*); Raadik 1996: 13; Koster 1997: 9 (*partim*); McDowall 2000: 1123 (*partim*); Raadik 2000: 5; Littlejohn 2001: 803; Raadik *et al.* 2001a: 9 (*partim*); Raadik *et al.* 2001b: 110 (*partim*); Unmack 2001: 1060 (*partim*); Allen *et al.* 2002: 111 (*partim*); Fields 2002; 3 (*partim*); Hammer 2002: 6; Koster 2003: 268 (*partim*); Hammer & Horne 2004: 2; Hammer & Walker 2004: 85; Romanowski 2004: 80 (*partim*); Kuiter 2005: 162, Fig. p163 (*partim*); Paxton *et al.* 2006: 408 (*partim*); McDowall 2006: 356 (*partim*); Threatened Species Section 2006: 44 (*partim*); Crook *et al.* 2008: 10; Hammer 2009: ii; Hammer *et al.* 2009: 3; Galeotti *et al.* 2010: 13 (*partim*); Saddlier *et al.* 2010: 3 (*partim*); Hammer *et al.* 2012: 63; Crook & Gillanders 2013: 207 (*partim*); Hammer *et al.* 2013: 70 (*partim*); King *et al.* 2013: 165 (*partim*); Kuiter 2013: 84 (*partim*), 8 lower images p. 86; Lintermans 2013c: 286 (*partim*); Bachmann *et al.* 2014: 1; Raadik 2014: 160 (*partim*).
Galaxiella pusilla 'west region'—Coleman *et al.* 2010: 1911; Unmack *et al.* 2012: 1; Coleman *et al.* 2013: 1821.
Galaxiella pusilla II—Unmack 2013: 43.
Galaxiella sp.–Lieschke *et al.* 2013a: 15; Lieschke *et al.* 2013b: 297.

Material examined.
Holotype. NMV A.31214-001, 29.2 mm SL, female, Surrey River, Wrights Swamp Road, Cobboboonee, Victoria, 38° 8' 48"S 141° 24' 55"E, R.A. Coleman, J.A. Coleman 2 August 2013.

Paratypes. Victoria: NMV A.31214-002, 20.7 mm SL, male, collected with holotype (Allotype); NMV A.31218-001 (20), 19.8–25.8 mm SL, Barwon River East Branch, Wickhams Road, Barwon Downs, 38° 28' 11"S 143° 44' 38"E, RAC, JAC, 17 July 2013; NMV A.31217-001 (2), 24.0–26.1 mm SL, Gosling Creek, Division Road, Murroon, 38° 26' 17"S 143° 49' 3"E, RAC, JAC, 28 June 2013; AMS I.46500-001 (2) female 21.5 mm SL and male 19.6 mm SL, NMV A.31211-001 (16), 17.8–23.4 mm SL, SAMA F. 14533 (1), female, 22.0 mm SL, SAMA F. 14534 (1), male, 19.2 mm SL, Bridgewater Lakes, Bridgwater Lakes Road, Tarragal, 38° 19' 13"S 141° 24' 16"E, RAC, JAC, 2 August 2013; NMV A.31215-001 (20), 16.7–25.9 mm SL, Darlot Creek tributary, Etterick Tyrendarra Road, Tyrendarra, 38° 9' 29"S 141° 46' 24"E, RAC, JAC, 27 June 2013; NMV A.31212-001 (1), 18.9 mm SL, Fitzroy River, T and W Road, Cobboboonee, 38° 4' 33"S 141° 25' 44"E, RAC, JAC, 2 August 2013; NMV A.31216-001 (20), 17.0–29.5 mm SL, Moyne River tributary, Woolsthorpe Heywood Road, Moyne, 38° 12' 48"S 142° 14' 24"E, RAC, JAC 28 June 2013; NMV A.31214-003 (18), 16.9–30.5 mm SL, collected with holotype, NMV A31213-001 (20), 18.9-27.5 mm SL, Crawford River, Condah Coleraine Road, Branxholme, 37° 52' 13"S 141° 43' 45"E, RAC, JAC, 27 June 2013.

Non-type material. South Australia: NMV A31222-001 (3) not measured, Death Hole Outlet Drain, Burkes Island, Homestead Road, Beachport, 37° 27' 50"S 140° 5' 55"E, RAC, E. Furlan, 15 April 2010; NMV A31219-001 (12) not measured , Hammerhead Lagoon, Piccaninne Ponds Road, Donovans, 38° 3' 1"S 140° 57' 15"E, RAC, E. Tsyrlin, 8 September 2008; NMV A31220-001 (6) not measured, Picks Swamp, ACI Road, Donovans, 38° 2' 33"S 140° 54' 43"E, RAC, E. Furlan, 12 April 2010; NMV A31221-001 (2) not measured, Reedy Creek Wilmont Drain, Konetta Road, Greenways, 37° 12' 2"S 140° 10' 20"E, RAC, E. Furlan, 14 April 2010; **Victoria:** NMV A30675-001 (20) not measured, Big Swamp, Henty Highway, Grampians National Park, 37° 18' 16"S 142° 11' 31"E, T.A. Raadik, 1 May 1996.

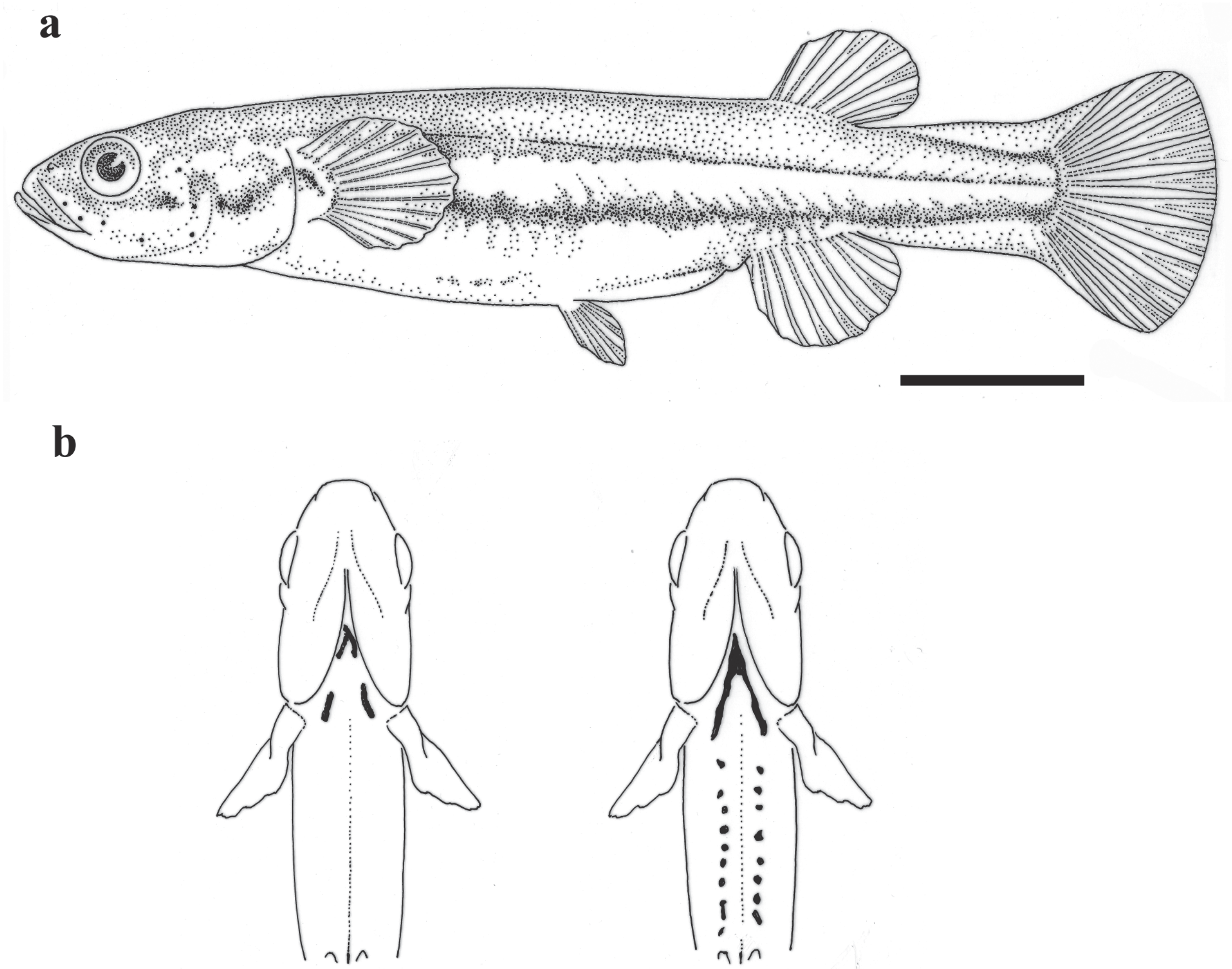


FIGURE 9. a) line drawing of *Galaxiella toourtkoourt* holotype, NMV A.31214-001, female, Surrey River, Wrights Swamp Road, Cobboboonee, Vic., 29.2 mm SL (R. Plant); scale bar = 5 mm; b) line drawing of typical anterio-ventral surface markings on *Galaxiella toourtkoourt* (left) and *Galaxiella pusilla* s.s. (right) (R. Plant).

Diagnosis. *Galaxiella toourtkoourt* is distinguished from other *Galaxiella* by: caudal fin rays usually 13 (12–15), anal fin rays usually 8 (7–10), pectoral fin rays usually 12 (8–13), usually 36 (34–38) vertebrae, 7 laterosensory pores in the preopercular-supramaxillary series. Adults very small (usually females 20.6–30.6 mm SL; males 16.7–24.0 mm SL); short caudal peduncle usually 19.6–21.9 %SL (females 17.2–23.8 %SL; males 17.9–24.4 %SL); origin of dorsal fin more or less in line with anal fin (particularly females) with horizontal distance between dorsal fin and anal fin origins usually 0.0–2.7 %SL (females -2.5–2.8; males -2.6–3.9). *Galaxiella toourtkoourt* adults with three longitudinal black stripes (easier to discern in adult males) and reduced markings on ventral surface (typically two to three black blotches at isthmus) (Fig. 9b).

Description. As for genus, except below. Based on 123 adult specimens, 16.7–30.6 mm SL, and 43 additional non-type specimens for meristic counts. See Table 2 for summary of meristic variation and Table 4 for frequencies of meristic values. Segmented dorsal fin rays 7 (5–8), of these 4 (1–5) branched; 4th dorsal fin ray longest (2nd–5th); segmented anal fin rays 8 (7–10), of these 5 (1–7) branched; 4th anal fin ray longest (3rd–6th); caudal fin rays 13 (12–15), of these 10 (5–11, excluding single outlier of 2) branched; segmented pectoral fin rays 13 (8–13); pelvic fin rays 5 (5–6); total gill raker total count on first gill arch (lower limb and upper limb) 17 (13–19), lower limb 11 (9–12) and upper limb 6 (3–7); vertebrae 36 (34–38).

See Figs. 3–4 and Tables 3 and 5 for summary of morphometric characters. Small, moderately elongate, mostly fusiform body with greatest depth usually in mid abdominal region (between pectoral and pelvic fins). Body depth at vent 15.2 (10.3–16.8) %SL females and 13.5 (12.8–15.8) %SL males, tapering to moderately short caudal peduncle 20.4 (17.2–23.8) %SL females and 22.7 (17.9–24.4) %SL males. Caudal peduncle deep 54.9 (38.5–60.1)

%LCP females and 43.3 (36.1–59.4) %LCP males. Dorsal and ventral trunk profiles moderately arched, flattened around head, narrowing posterior to vent.

Head short (females 27.8 (20.6–28.4) %SL; males 28.3 (22.4–28.3) %SL), broad (females 62.2 (60.2–87.8) %HL; males 56.8 (40.3–80.4) %HL) and similar depth (females 61.3 (60.2–90.5) %HL; males 60.2 (58.6–84.7) %HL), snout round and short (females 21.0 (13.9–25.4) %HL; males 20.9 (16.0–24.3) %HL). Eye diameter large (females 24.4 (24.4–36.4) %HL; males 30.1 (29.3–37.2) %HL), positioned high in head, typically upper margin just below, or level with, dorsal head profile; interorbital generally broad (females 38.6 (35.5–52.0) %HL; males 37.1 (30.0–52.6) %HL) and flat. Mouth small, extending posterio-ventrally from near tip of snout to almost vertically at anterior margin of eye. Lower jaw slightly shorter (females 26.9 (18.8–27.3) %HL; males 25.5 (14.0–26.7) %HL) than upper jaw (females 27.7 (20.2–28.8) %HL; males 26.7 (15.2–29.3) %HL), gape width moderate (females 26.9 (23.3–38.4) %HL; males 23.2 (20.8–33.6) %HL) and gape depth slightly shorter in comparison (females 22.7 (17.4–30.5) %HL; males 24.3 (14.6–27.9) %HL). Jaws without enlarged lateral canines; mesopterygoidal teeth few but strong; lingual teeth well developed. Gill rakers generally long, slender and pointed, sometimes rounded. Seven pores in preopercular-supramaxillary series with bow-shape cluster of three closest to jaw; submandibular sensory pores absent.

Fins small, lacking spines, membranous and predominantly rounded. Length of anal fin base (females 10.7 (9.2–14.2) %SL; males 10.5 (9.4–15.3) %SL) greater than dorsal fin base (females 9.1 (6.6–10.9) %SL; males 8.2 (7.2–11.7) %SL) and anal fin of similar length (females 16.1 (12.6–20.0) %SL; males 15.8 (13.8–19.6)%SL) to dorsal fin (females 16.6 (12.6–17.3) %SL; males 14.4 (12.5–18.8) %SL). Dorsal fin and anal fin origins usually almost in line, with fin origin setback more or less zero (females 0.4 (-2.5–2.8) %SL; males 1.5 (–2.6–3.9) %SL), though occasionally dorsal fin origin marginally anterior to anal fin origin. Pelvic fin to anal fin distance 20.8 (18.6–26.1) %SL females and 20.0 (17.3–22.4) %SL males, pelvic fins narrow and shorter (females 11.9 (9.3–12.4) %SL; males 11.5 (8.9–13.0) %SL) than dorsal and anal fins, positioned mid-way along ventral surface of body. Pectoral fins with broad, fleshy base, inserted high on lateral surface of body, approximately same level as tip of snout, round to paddle-shaped, and similar length (females 11.9 (9.8–15.1) %SL; males 13.5 (9.3–15.9) %SL) or slightly larger than, pelvic fins. Caudal fin rounded, moderate length (females 15.9 (12.3–27.3) %SL; males 18.3 (13.7–20.9) %SL), generally more broad than long, and vertical width of expanded rays greater than depth of caudal peduncle. Caudal peduncle flanges deep and distinct, extending forward almost to posterior end of anal fin base. Well-developed fleshy keel present (females 16.8 (10.5–19.6) %SL; males 15.4 (11.3–17.9) %SL), extending mid-ventrally from between pelvic fins to vent, maximum depth 2.8 (1.6–3.4) %SL females and 2.6 (1.6–3.3) %SL males.

Size. The smallest species in the genus *Galaxiella*, with females recorded to 42 mm TL; (commonly to 27–32 mm TL) and males recorded to 34 mm TL (commonly to 25–28 mm TL). Also the smallest species in the Galaxiidae (e.g. McDowall & Waters 2004).

Colour in life. The dorsal and upper sides of body are generally pale olive-brown (darker towards dorsal margin) and the ventral and lower sides silvery-white (Fig. 10a). The gills are visible as a large red-pink patch through gill covers on the sides of the head. Adults possess three continuous to semi-continuous, horizontal black stripes along the lateral surfaces of the trunk from near the eye to the origin of the caudal fin (tending to be more distinct and complete in males). Stripes are generally fainter and stippled in females, with the upper two stripes difficult to discern (particularly the dorsal stripe). Adult males possess a bright orange-red stripe between the lower two black stripes, which in females is generally thin and bright silver, gold, mauve or on rare occasions, pale orange. The eyes are mostly silvery-gold, with patches of bright orange in line with the coloured body stripe (of the same colour in males). In adults, the ventral surface has few markings except two to three black blotches at the isthmus and rarely with faint, discontinuous dots between the ventral surface of head and the pelvic fin bases (Fig. 9b). Juveniles lack the distinct striped patterns and colour of adults, are predominantly pale olive dorsally to beige on the upper sides and silvery-white ventrally. The eyes of juveniles are silvery-gold, with a crenulated stripe of similar colour along the sides.

Colour of preserved material. The body is creamy beige with darker stippling along the dorsal surface (particularly around head) and paler on the lower sides and ventral surface. Adults have three continuous to semi-continuous, horizontal black stripes along the lateral surfaces of the trunk from near the eye to the origin of the caudal fin and tending to be more distinct and complete in males. The stripes are generally fainter and stippled in females, with the upper two stripes difficult to discern (particularly the dorsal stripe). Coloured stripes are absent and the eyes are black. The ventral surface of adults has few markings, except two to three black blotches at the isthmus and rarely with faint, discontinuous dots between the ventral surface of the head and the pelvic fin bases.

FIGURE 10. a) *Galaxiella toourtkoourt* male (upper) and female (lower) from Narrow Neck Drain, Hatherleigh, South Australia (Photo by M. Hammer), b) *Galaxiella pusilla* s.s. male (lower) and female (upper) from Tuerong Creek, Moorooduc, Victoria (Photo by R. Kuiter).

Sexual Dimorphism. Sexes exhibit dimorphism in colouration and morphological characters which, along with that expressed in *Galaxiella pusilla* sensu stricto (s.s.), is a unique feature within *Galaxiella* and the Galaxiidae. Whilst differences in many of the morphological characters measured were evident, key differences between the sexes of adult fish are: females are longer and deeper bodied than males, and the genital papillus in

males is low and projects posteriorly slightly further than the vent. The female genital papillus is a distinct fleshy mound. Colour differences between the sexes of adult fish are: males have a bright orange-red lateral body stripe (generally thin and bright silver, gold or mauve in females) and three distinct black lateral body stripes, which are more difficult to discern in females, particularly the most dorsal stripe (Fig. 10a).

Etymology. Pronounced "Too-urt Koo-urt" (or Tu-urt Ku-urt), from the Australian indigenous language groups Tjapwurrung, Korn Kopan noot, and Peekwurrung, meaning 'little fish in freshwater'. Recommended standard name as 'Little galaxias' based on it being the smallest species in the Galaxiidae (e.g. McDowall &Waters 2004) and consistency with 'little' in the indigenous language meaning.

Genetics. Microsatellite and mtDNA analysis of this species can be found in Coleman *et al.* (2010; 2013; taxon 'west region'). Allozyme and mtDNA data are presented in Unmack *et al.* (2012).

Distribution. Known from coastal south-eastern mainland Australia, from the upper Barwon River system near Barwon Downs, Victoria, west to the Cortina Lakes, near the Coorong, South Australia (Fig. 11). Its current known range does not overlap that of its sister species *Galaxiella pusilla* s.s.

Habitat. Generally found in swamps, wetlands, shallow lakes, billabongs, small creeks and artificial earthen drains (Fig. 12) at low elevation (mean 100 m above sea level, typically 22–176 m above sea level). Habitats are mostly shallow (mean maximum depth 1.1 m, typically 0.5–2.0 m), with still to low water velocities (or often backwaters in faster flowing conditions) and partial shading (mean 27 %, typically 5–50 % surface cover). The substrate tends to be dominated by fine clay (mean 34 %, typically 0–50 %) and silt (mean 43 %, typically 25–50 %) and occasionally coarser (particularly sand) with deposits of coarse particulate organic matter (mean 25 %, typically 0–40 % cover). Frequently captured where the vegetation cover is dense and consists primarily of emergent (mean 36 %, typically 15–50 % cover) and submerged (mean 17 %, typically 0–23 % cover) aquatic species. Measurements of water quality at the time of collection suggest that *G. toourtkoourt* can withstand a broad range of conditions, being recorded at water temperatures of 5.2–26.9 ºC, dissolved oxygen levels of 20–263 % saturation, pH of 5.3–9.3, water electrical conductivity of 94–13,620 µS/cm and turbidity of 1–96 NTU.

Conservation status. Formerly part of *Galaxiella pusilla* s.l., which is currently listed as 'vulnerable' nationally (*Environment Protection and Biodiversity Conservation (EPBC) Act 1999*) and internationally (IUCN Red List of Threatened Species, Wager 1996). *Galaxiella pusilla* s.l. is currently considered threatened in South Australia where it is also listed as 'vulnerable' (Hammer *et al.* 2009). In the state of Victoria, *G. toourtkoourt*, (listed as '*Galaxiella* sp.') is considered threatened and has been categorised as 'vulnerable' (DSE 2013). According to the IUCN criteria (IUCN 2012), from data presented here it should be considered nationally 'endangered' based on the following criteria: B2a, b(i,ii,iii,iv), c(ii,iii), that relate to an area of occupancy <500km^2, severe population fragmentation, continuing decline in the extent of occurrence, area of occupancy, area/extent and/or quality of habitat, number of locations or subpopulations, and extreme fluctuation in the area of occupancy and number of locations or subpopulations. Habitat loss and fragmentation, and negative interactions with invasive fishes (particularly the poecilid *Gambusia holbrooki*), are considered major threats to its long-term survival (Koster 2003; Saddlier *et al.* 2010; Coleman *et al.* in review).

General biology. Non-migratory, freshwater species, that is occasionally found in inland slightly saline waters. The spawning period is possibly during late autumn to spring, but may be variable (Beck 1985; Romanowski 2004) and although some individuals may overlap breeding seasons, are thought to be predominantly a semelparous species, living one year and dying soon after spawning (Romanowski 2004). Where present, it can occur in high densities, although there may be substantial seasonal and inter-annual variability in population abundances (Romanowski 2004; Coleman *et al.* in review). It has developed adaptations to surviving extended seasonal periods of habitat drying, aided via a capacity for air-breathing and the use of moisture retaining habitat features such as vegetation cover and crayfish burrows (Beck 1985; Romanowski 2004; Coleman *et al.* in review). It is likely to exist within a metapopulation structure or a 'population of sub-populations', where persistence of the population as a whole is facilitated by multiple interconnected sub-populations (*viz.* Levins 1969; Hanski 1998). Natural wetting and drying cycles typical of their habitats (and other environmental factors) can lead to large fluctuations in inter-annual population densities, emphasising the importance of habitat inter-connectivity across the landscape to maintain a balance between local population extinctions and recolonization (Coleman *et al.* in review). Wetting and drying cycles are also likely to be important for enhancing food resources and reducing competition and predation pressures (Coleman *et al.* in review).

It is often collected with native fish species, particularly *Nannoperca australis* (65 % frequency), *Galaxias maculatus* (12 % frequency), and burrowing crayfish (e.g. *Engaeus* spp., *Geocharax* spp.) (18 % frequency). Also

often found with the alien fish species *Gambusia holbrooki* (29 % frequency). Aquatic macroinvertebrates found to occur with *G. toourtkoourt* include *Paratya australiensis* (24 % frequency), Notonectidae (35 % frequency), Amphipoda (20 % frequency), Belostomatidae (18 % frequency), Corixidae (24 % frequency), Coleoptera (18 % frequency), Odonata (37% frequency), Gastropoda (26 % frequency). It is sometimes found with clusters of trematode cysts within the head region that can cause head enlargement (Coleman 2014).

Remarks. *Galaxiella toourtkoourt* differs from *Galaxiella pusilla* s.s. primarily by a combination of the following characters: less vertebrae; slightly shorter caudal peduncle; less pronounced dorsal fin-anal fin setback, especially in females; adults are smaller. In preserved material, reduced markings on the ventral surface of adults, typically two to three black blotches at the isthmus (Fig. 9b), also differentiate it from *G. pusilla* adults. The shorter dorsal fin-anal fin setback in *G. toourtkoourt* distinguishes it from *Galaxiella nigrostriata* and *Galaxiella munda* as well. Additional differences to *G. nigrostriata* are: less caudal and anal fin rays; one more longitudinal black lateral body stripe; and, less laterosensory pores in the preopercular-supramaxillary series. Also differs from *G. munda* by being shorter overall, having less caudal and anal fin rays, more pectoral fin rays, less vertebrae, and possessing longitudinal dark lateral body stripes in adults.

Other, smaller, differences between *Galaxiella toourtkoourt* and *G. pusilla* s.s. have been noted, which may be of secondary taxonomic importance: 1) caudal peduncle flanges often appear to only extend to near the posterior end of the anal fin base in *G. toourtkoourt*, but frequently extend to near the posterior end of both the anal fin base and dorsal fin base in *G. pusilla* s.s.; 2) subtle difference in the positioning of the eye may also be present, whereby the upper margin of the eye appears to be more consistently just below, or level with, the dorsal head profile in *G. toourtkoourt*, while it is frequently level with, or extends slightly above, the dorsal head profile in *G. pusilla* s.s.; and, 3) during laboratory experiments, when single populations of live fish were held in aquaria at the same time, *G. toourtkoourt* individuals were distinctly more pale and yellowish than *G. pusilla* s.s.—it is unknown how consistent this last potentially distinguishing feature is across populations and environmental conditions.

Galaxiella pusilla Mack, 1936

Dwarf galaxias

Tables 2 to 3; Figs. 3 to 4, 9b, 10b, 11 and 13

Galaxias pusillus Mack, 1936: 101 (holotype NMV A.97, paratypes NMV A.98 (1), NMV A.388–390 (3); type locality 'Cardinia Creek, about 30 miles east of Melbourne, Victoria')—Massola 1938: 129; Whitley 1939: 268; Stokell 1945: 126; Shipway 1953; 173; McDowall 1971: 36; Dixon 1972: 121; McDowall 1973: 193; Andrews 1976 (*partim*): 324.

Galaxias pucillus—Mack, 1936: 101 (misprint in legend of Fig. 2).

Brachygalaxias pusillus—Scott 1942: 55; Stokell 1954: 412; Whitley 1956a: 34; 1956b: 39 (*partim*); 1957: 6; 1960: 29; 1964: 35; Scott 1966: 246 (*partim*); Frankenberg 1966a: 162; 1966b: 32; 1967: 227 (*partim*); 1968: 172; Lake 1971: 20 (*partim*); Scott 1971: 3 (*partim*); Dixon 1972: 121; Frankenberg 1974: 119; Pollard 1980: 348 (*partim*).

Brachygalaxias pusillus pusillus—Munro 1957: 18 (*partim*); Frankenberg 1969: 185 (*partim*); Frankenberg 1971: 94 (*partim*); McDowall1973: 193; Scott 1971: 3.

Brachygalaxias pusillus flindersiensis Scott, 1971: 6 (holotype: QVM 1969:5:35 (previously 1969.5.25a); paratypes: AMS I.16158-001 (1), BMNH 1972.1.27.1 (1), QVM 1969:5:34 (12); type locality: 'Lackrana, Flinders Island, Furneaux Group, Bass Strait');–Green 1974: 3; Frankenberg 1974: 114.

Brachygalaxias pusillus tasmaniensis Scott, 1971: 3 (holotype: QVM 1971.5.48 (listed in error as 1970/5/25a in original description); paratypes: AMS I.16159-001 (1), BMNH 1972.1.27.2 (1), QVM 1971:5:50 (11); type locality 'from a soak from a dam flowing towards the coast (northward) on *Marengo*, Waterhouse Estate, Dorset, Tasmania'); Green 1974: 3; Frankenberg 1974: 114.

Galaxiella pusilla—McDowall 1978b: 309; Lake 1978: 26 (*partim*); Backhouse & Vanner 1978: 129 (*partim*); McDowall 1980: 55 (*partim*); McDowall & Frankenberg 1981: 552 (*partim*); Backhouse 1983: 6 (*partim*); Cadwallader & Backhouse 1983: 75 (*partim*); Humphries 1983: 4 (*partim*); Johnson *et al.* 1983: 50; Last *et al.* 1983: 201 (*partim*); Merrick & Schmida 1984: 98 (*partim*); Humphries 1986: 133; Allen 1989: 44 (*partim*), 89; Berra & Allen 1989: 293 (*partim*); Breen *et al.* 1989: 1; Paxton *et al.* 1989: 179 (*partim*); Fulton 1990: 39 (*partim*); Koehn & Morison 1990: 20 (*partim*); Andrews 1991: 59 (*partim*); Pen *et al.* 1993: 848 (*partim*); Humphries 1995: 1159 (*partim*); Unmack & Paras 1995: 398 (*partim*); Westbury 1995: 1 (*partim*); Chilcott & Humphries 1996: 147 (*partim*); McDowall & Fulton 1996: 70 (*partim*); Koster 1997: 9 (*partim*); Raadik & Zampatti 1998: 6; McDowall 1999: 936; 2000: 1123 (*partim*); Waters *et al.* 2000: 794; Unmack 2001: 1060 (*partim*); Allen *et al.* 2002: 111 (*partim*); Fields 2002: 3 (*partim*); Koster 2003: 268 (*partim*); McDowall & Waters 2004: 24; Romanowski 2004: 80 (*partim*); Kuiter 2005: 162 (*partim*); Hardie *et al.* 2006: 238; McDowall 2006: 356 (*partim*); Paxton *et al.* 2006: 408 (*partim*); Raadik 2006: 139; Threatened Species Section 2006: 44 (*partim*); Galeotti *et al.* 2010: 13 (*partim*); Saddlier *et al.* 2010: 3 (*partim*); Stoessel 2010: 2; McDowall & Burridge 2011:

9; Burridge *et al.* 2012: 313; Hammer *et al.* 2012: 62; Crook & Gillanders 2013: 207 (*partim*); Hammer *et al.* 2013: 70 (*partim*); King *et al.* 2013: 165 (*partim*); Koehn & Kennard 2013: 91; Kuiter 2013: 84 (*partim*), images pp 84-85, images top p. 86, images p. 87; Lieschke *et al.* 2013a: 15; Lieschke *et al.* 2013b: 235; Lintermans 2013c: 286 (*partim*); Raadik 2014: 160 (*partim*); Galeotti *et al*. 2015: 189.

Galaxiella pusilla 'east region'—Coleman *et al.* 2010: 1911; Unmack *et al.* 2012: 1; Coleman *et al.* 2013: 1821.

Material examined.

Holotype. NMV A.97, 22.3 mm SL [now partially dried, particularly anterior end], male, Cardinia Creek, about 30 miles east of Melbourne, Victoria, A. Massola, 22 May 1936.

Paratypes. Victoria: NMV A. 98 and A.388-90, collected with holotype.

Non-type material. Victoria: NMV A.31230-001 (20), 20.9–31.2 mm SL, Cobblers Creek, Forge Creek Road, Bairnsdale, 37° 51' 17"S 147° 36' 19"E, RAC, JAC, 26 July 2013; NMV A.31229-001 (20), 18.1–28.7 mm SL, Perry River, Princes Highway, Delvine, 37° 55' 17"S 147° 16' 31"E, RAC, JAC, 26 July 2013; NMV A.31226-001 (20), 22.3–31.8 mm SL, Moe Main Drain, Mitchells Road, Moe, 38° 10' 22"S 146° 14' 52"E, RAC, JAC, 29 July 2013; NMV A.31227-001 (23), 23.5–32.5 mm SL, Morwell River, Princes Freeway, Morwell, 38° 13' 40"S 146° 21' 39"E, RAC, JAC, 29 July 2013; NMV A.31228-001 (20), 19.2–28.8 mm SL, Merriman Creek, Merton-Vale Road, Willung, 38° 14' 54"S 146° 49' 26"E, RAC, JAC, 19 July 2013; NMV A.31223-001 (20), 20.0–32.3 mm SL, Dandenong Creek tributary, Brady Road, Endeavour Hills, 37° 57' 37"S 145° 14' 2"E, RAC, JAC, 5 July 2013; NMV A.31225-001 (20), 21.1–28.1 mm SL, King Parrot Creek, Greenshield Road, Drouin, 38° 10' 27"S 145° 52' 7"E, RAC, JAC, 5 July 2013; NMV A.31224-001 (20), 24.2–32.7 mm SL, Tuerong Creek, Old Moorooduc Road, Moorooduc, 38° 16' 23"S 145° 4' 16"E, RAC, JAC, 5 July 2013. **Tasmania:** NMV A.31231-001 (10) not measured, Icena Creek, Browns Bridge Road, Gladstone, 40° 58' 12"S 148° 9' 23"E, RAC, M.L. Coleman, 27 May 2008; NMV A.31232-001 (9) not measured, Big Waterhouse Lake, Homestead Road, Waterhouse, 40° 53' 33"S 147° 36' 53"E, RAC, M.L. Coleman, 28 May 2008, and same location, RAC, H.T. Coleman, E. Tsyrlin, 1 July 2009.

Diagnosis. *Galaxiella pusilla* s.s. is distinguished from other *Galaxiella* by: caudal fin rays usually 13 (11–15), anal fin rays usually 8 (7–10), pectoral fin rays usually 12 (10–13), usually 39 (36–41) vertebrae, 7 laterosensory pores in preopercular-supramaxillary series. Adults small (usually females 21.4–32.7 mm SL; males 18.1–28.2 mm SL); caudal peduncle length moderate usually 21.3–23.8 %SL (females 18.4–25.5; males 20.3–26.0); origin of dorsal fin distinctly posterior to anal fin (particularly males), with horizontal distance between dorsal fin and anal fin origins usually 1.4–3.8 %SL (females 0.0–3.8; males 0.52–5.67). *Galaxiella pusilla* s.s. adults with three longitudinal black stripes (easier to discern in adult males) and distinct markings on ventral surface (typically 'v'-shaped dark mark originating on isthmus and extending as two parallel dotted lines (sometimes discontinuous) to the pelvic fin bases) (Fig. 9b).

Description. As for genus, except as below. Based on 163 adult non–type specimens, 18.1–32.7 mm SL, and 19 additional non–type specimens for meristic counts. See Table 2 for summary of meristic variation and Table 4 for frequencies of meristic values. Segmented dorsal fin rays 7 (6–8, holotype 6), of these 4 (0–5, holotype 4) branched; 4th dorsal fin ray longest (2nd–5th); segmented anal fin rays 8 (7–10, holotype 8), of these 5 (0–7) branched; 4th anal fin ray longest (3rd–7th); caudal fin rays 13 (11–15, holotype 13), of these 9 (4–11, excluding a single outlier of 1, holotype 9) branched; segmented pectoral fin rays 12 (10–13, holotype 13); pelvic fin rays 5 (4–5, holotype 5); total gill raker total count on first gill arch (lower limb and upper limb) 16 (13–20), lower limb 10 (8–13) and upper limb 6 (4–7); vertebrae 39 (36–41, holotype 38).

See Figs. 3–4 and Tables 3 and 5 for summary of morphometric characters. Small, moderately elongate and mostly fusiform body with greatest body depth usually in mid abdominal region (between pectoral and pelvic fins). Body depth at vent 13.8 (12.0–15.5) %SL females and 13.6 (11.8–15.4) %SL males, tapering to moderately long caudal peduncle (females 22.2 (18.4–25.5) %SL; males22.9 (20.3–26.0) %SL). Caudal peduncle moderately deep (females 43.8 (34.3–53.6) %LCP; males 43.2 (36.9–52.9) %LCP). Dorsal and ventral profiles moderately arched, flattened in head region and narrowing behind vent.

Head short (females 23.0 (19.7–26.8) %SL; males 23.8 (19.7–26.9) %SL), broad (females 70.4 (61.4–87.5) %HL; males 65.2 (56.6–81.9) %HL) and similar depth (females 68.1 (55.9–83.1) %HL; males 66.8 (60.1–77.7) %HL). Snout rounded and short (females 20.9 (13.6–26.9) %HL; males 20.7 (15.9–24.6) %HL). Eye diameter large (females 30.6 (25.3–36.4) %HL; males 32.0 (28.1–35.9) %HL), positioned high in head with upper margin typically level with dorsal head profile or slightly raised above. Interorbital generally broad (females 44.8 (37.2–

58.9) %HL; males 43.4 (36.7–59.3) %HL) and flat. Mouth small, slanting posterio-ventrally from tip of snout to almost in line with anterior margin of eye. Lower jaw slightly shorter (females 22.4 (16.6–26.9) % HL; males 22.1 (15.9–27.4) %HL) than upper jaw (females 24.2 (18.5–31.2) %HL; males 23.7 (18.3–28.6) %HL). Gape width moderate (females 27.8 (21.3–38.9) %HL; males 25.6 (21.1–34.2) %HL) and depth slightly shorter (females 22.4 (14.9–27.1) %HL; males 21.6 (14.0–26.1) %HL) than width. Jaws without enlarged lateral canines; mesopterygoidal teeth few but strong; lingual teeth well developed. Gill rakers generally long, slender and pointed, sometimes rounded. Seven pores in preopercular-supramaxillary series with bow-shape cluster of three closest to jaw; submandibular sensory pores absent.

Fins small, lacking spines, membranous and predominantly rounded. Length of anal fin base (females 11.0 (9.7–13.3) %SL; males 12.1 (10.2–14.5) %SL) greater than dorsal fin base (females 8.3 (6.5–10.2) %SL; males 8.7 (6.8–10.6) %SL). Anal fin similar length (females 15.9 (14.1–19.1) %SL; males 17.2 (15.2–19.9) %SL) to dorsal fin (females 14.6 (12.8–17.2) %SL; males 15.1 (12.7–17.7) %SL). Dorsal fin origin distinctly posterior to anal fin origin (DF–AF setback in females 2.2 (0.0–3.9) %SL; males 3.1 (0.5–5.7) %SL). Pelvic fin to anal fin distance ranges from 20.6 (17.9–24.0) %SL females and 18.8 (16.9–22.8) %SL males. Pelvic fins narrow and shorter (females 9.9 (8.0–11.3) %SL; males 10.4 (8.7–12.2) %SL) than dorsal and anal fins, positioned mid-length along ventral surface of body. Pectoral fins with broad, fleshy base, inserted high, approximately same level as tip of snout, round (paddle-like), of similar length to, or slightly larger (females 11.8 (9.6–14.0) %SL; males 12.5 (10.7–15.2) %SL) than pelvic fins. Caudal fin rounded, moderate length (females 16.1 (9.3–20.2) %SL; males 16.8 (10.5–20.2) %SL), generally broader than long, and vertical width of expanded rays greater than depth of caudal peduncle. Caudal peduncle flanges broad, distinct, extending back almost to posterior ends of anal and dorsal fin bases. Well-developed fleshy keel present mid-ventrally between pelvic fins, extending to vent (females 15.1 (12.1–19.1) %SL; males 14.5 (11.2–17.6) %SL) with maximum depth 2.0 (1.3–2.9) %SL in females and 1.9 (0.8–3.0) %SL in males.

Size. Females recorded to 47 mm TL, commonly to 30–36 mm TL. Males recorded to 37 mm TL, commonly to 27–31 mm TL.

Colour in life. The dorsal and upper sides of the body are generally pale olive-brown (darker along the dorsal margin) and the ventral and lower sides silvery-white (Fig. 10b). The gills are visible as a large red-pink patch through the gill covers on the sides of the head. Adults possess three continuous to semi-continuous, horizontal black stripes along the lateral surface of the trunk, from near the eye to the origin of the caudal fin. In males the black lines are more distinct and complete, and in females the lines are generally fainter, stippled and the upper two stripes more difficult to discern (particularly the dorsal stripe). Adult males possess a bright orange-red stripe between the lower two black stripes, which in females is generally thin and bright silver, gold, pale mauve or on rare occasions, pale orange. The eyes are mostly silvery-gold, with patches of bright orange in line with the coloured body stipe (of the same colour in males). On the ventral surface of adults is a distinct v-shaped black mark often present on the isthmus, which frequently extends posteriorly as two parallel dotted lines to near the pelvic fins (Fig. 9b). The ventral markings more distinct in males, and the dotted lines are occasionally absent or discontinuous. The v-shape marking is rarely reduced to a few irregular blotches in females. Juveniles lack the distinct striped patterns and colour of adults, are predominantly pale olive dorsally to beige on the upper sides and silvery-white ventrally. The eyes of juveniles are silvery-gold and have a similar colour to the crenulated stripe along the sides.

Colour of preserved material. The body is creamy beige, with darker stippling along the dorsal surface (particularly around head) and paler on the lower sides and ventral surface. Adults have three continuous to semi-continuous, horizontal black stripes along the lateral surfaces of the trunk from near eye to the origin of the caudal fin and tending to be more distinct and complete in males. The stripes are generally fainter and stippled in females, with the upper two stripes difficult to discern (particularly the dorsal stripe). Coloured stripes are absent and the eyes are black. The ventral surface of adults has a distinct v-shaped black mark that is often present on the isthmus and frequently extends posteriorly as two parallel dotted lines to near the pelvic fins. The ventral markings more distinct in males and the dotted lines are occasionally absent or discontinuous. The v-shape marking is rarely reduced to a few irregular blotches in females.

Sexual Dimorphism. Whilst differences in many of the morphological characters measured were evident, key features that summarise sexual dimorphism in *G. pusilla* s.s. adults include: males smaller than females; females tend to be deeper bodied that males; male genital papillus is low and projects slightly more than the vent, while the

female genital papillus is a distinct fleshy mound. Colour differences between the sexes of adult fish are: males have a bright orange-red lateral body stripe and three distinct black lateral body stripes on the side, females lack the orange-red lateral body stripe (generally thin and bright silver, gold or mauve in females) and the three black stripes are more difficult to discern, particularly the most dorsal stripe (Fig. 10b).

Etymology. The name *pusilla* is from the Latin *pusillus* (as originally used by Mack, 1936), meaning 'very small'. Recommended standard name as 'dwarf galaxias'.

Genetics. Microsatellite and mtDNA analysis of this species can be found in Coleman *et al.* (2010; 2013; taxon 'east region'). Allozyme and mtDNA data are presented in Unmack *et al.* (2012).

Distribution. Known in coastal south-eastern mainland Australia, from the Mitchell River Basin near Bairnsdale, west to Dandenong Creek near Melbourne in Victoria, Flinders Island in Bass Strait and north-eastern and north-western Tasmania (Fig. 11). A translocated population (sourced in the early-mid 1990s from Tirhatuan Wetland, Rowville, in the Dandenong Creek catchment) exists in the La Trobe University wildlife reserve, Bundoora, in the Yarra River catchment (Westbury 1995). Its current known range does not overlap that of *G. toourtkoourt*.

Habitat. Generally found in swamps, wetlands, shallow lakes, billabongs, small creeks and artificial earthen drains (Fig. 13) at very low elevation (mean 54 m above sea level, typically 25–72 m above sea level). Habitats are mostly shallow (mean maximum depth 1.1 m, typically 0.5–1.5 m), with still to low water velocities (or often backwaters in faster flowing conditions) and substantial shading (mean 36 %, typically 10–60 % surface cover). The substratum composition tends to be dominated by fine clay (mean 33 %, typically 10–50 %) and silt (mean 43 %, typically 30–50 %) and occasionally coarser (particularly sand) with deposits of coarse particulate organic matter (mean 31 %, typically 10–60% cover). Frequently captured where the vegetation cover is dense and consists primarily of emergent (mean 45 %, typically 24–73 % cover) and submerged (mean 12 %, typically 0–13 % cover) aquatic species. Measurements of water quality at the time of collection suggest that this species can withstand a broad range of conditions, at least, water temperatures of 5.8–24.8 ^{o}C, dissolved oxygen levels of 18–130 % saturation and turbidity between 1–133 NTU. The range for pH measurements was 5.0–7.8 and the range for water electrical conductivity was 36–3,070 µS/cm.

Conservation status. *Galaxiella pusilla* s.l. is currently listed as 'vulnerable' nationally (*EPBC Act 1999)* and internationally (IUCN Red List of Threatened Species, Wager 1996). *Galaxiella pusilla* s.s. should be retained as a nationally threatened taxon, but considered more threatened than previously thought as its newly defined mainland distribution is now approximately 40% of that previously known for *G. pusilla* s.l. According to the IUCN criteria (IUCN 2012) it should qualify as 'endangered' based on the following criteria: B2a, b(i,ii,iii,iv), c(ii,iii),), that relate to an area of occupancy <500km^2, severe population fragmentation, continuing decline in the extent of occurrence, area of occupancy, area/extent and/or quality of habitat, number of locations or subpopulations, and extreme fluctuation in the area of occupancy and number of locations or subpopulations. This is consistent with the conservation status proposed by the State of Victoria in Australia for 'eastern region' populations of *Galaxiella pusilla* s.l. (DSE 2013). Habitat loss and fragmentation, and negative interactions with invasive fishes (particularly *Gambusia holbrooki*) remain as major threats to their long-term survival (Koster 2003; Saddlier *et al.* 2010; Coleman *et al.* in review).

General Biology. A non-migratory, freshwater species, occasionally found in inland slightly saline waters. The observed spawning period is between late autumn to spring and can be variable (Backhouse & Vanner 1978; Humphries 1986; Coleman *et al.* in review). It is predominantly an annual species that dies soon after spawning (Massola 1938; Humphries 1986; Pen *et al.* 1993; Romanowski 2004; Coleman *et al.* in review). Where present, it can occur in high densities, although there may be substantial seasonal and inter-annual variability in population abundances (Coleman *et al.* in review). It has developed adaptations to surviving extended periods of habitat drying, aided via a capacity for air-breathing and the use of moisture retaining habitat features such as vegetation cover and crayfish burrows (Coleman *et al.* in review). It is likely to exist with a metapopulation structure or a 'population of sub-populations', where persistence of the population as a whole is facilitated by multiple interconnected sub-populations (*viz.* Levins 1969; Hanski 1998). Natural wetting and drying cycles typical of their habitats (and other environmental factors) can lead to large fluctuations in inter-annual population densities, emphasising the importance of habitat inter-connectivity across the landscape to maintain a balance between local population extinctions and recolonization (Coleman *et al.* in review). Wetting and drying cycles are also likely to be important for enhancing food resources and reducing competition and predation pressures (Coleman *et al.* in review).

It is often collected with native fish species, particularly *Nannoperca australis* (69 % frequency), *Galaxias maculatus* (12 % frequency), and *Anguilla australis* (10 % frequency). Exotic fish species often collected with *G. pusilla* are *Gambusia holbrooki* (39 % frequency) and *Carrasius auratus* (12 % frequency). Other aquatic life that frequently occurs with *G. pusilla* include *Paratya australiensis* (19 % frequency), Notonectidae (40 % frequency), Amphipoda (10 % frequency), Belostomatidae (25 % frequency), Corixidae (15 % frequency), Coleoptera (10 % frequency), Odonata (44 % frequency), and Gastropoda (10 % frequency). It is sometimes found with clusters of trematode cysts within the head region that can cause head enlargement (Coleman 2014).

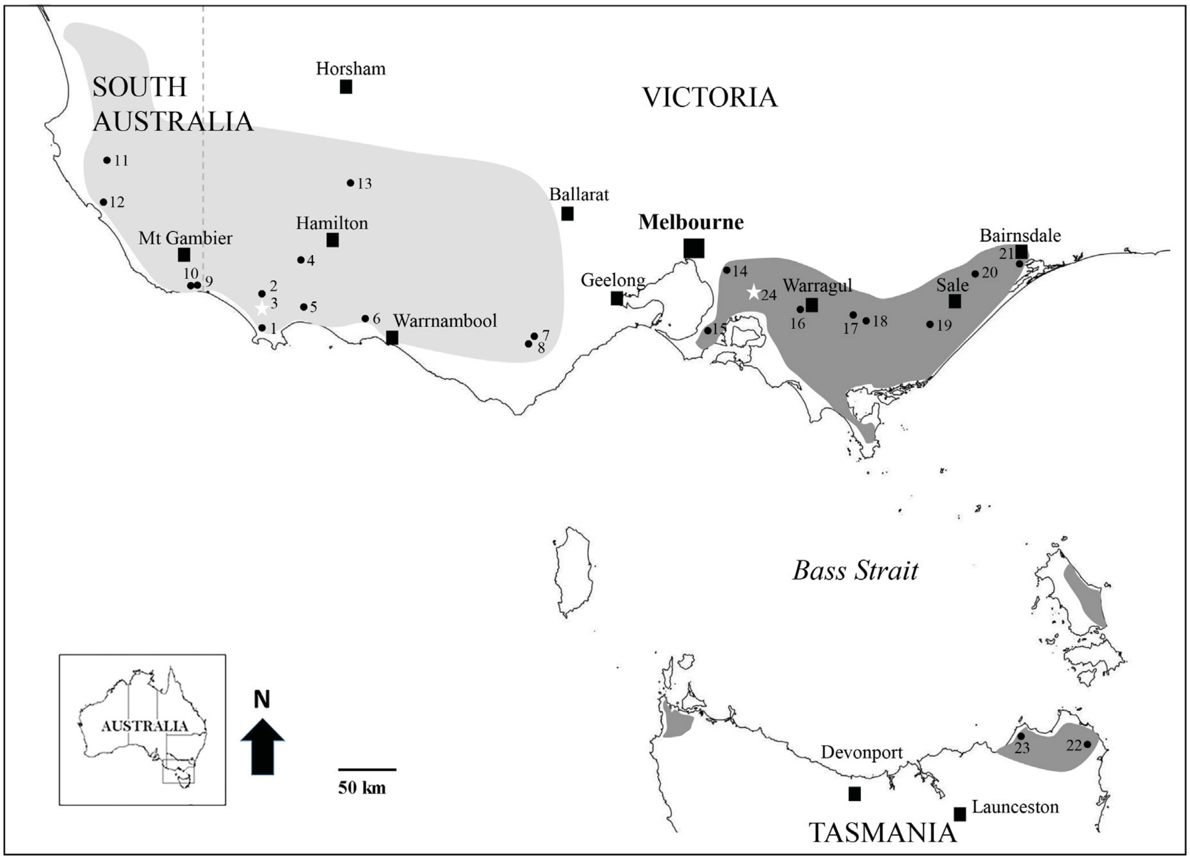


FIGURE 11. Collection sites and approximate current geographic distribution of *Galaxiella toourtkoourt* (light grey) and *Galaxiella pusilla* s.s. (dark grey). See Table 1 for site numbers and site details. Type localities are indicated by a white star.

Remarks. *Galaxiella pusilla* s.s. differs from *Galaxiella toourtkoourt* primarily by a combination of the following characters: more vertebrae; slightly longer caudal peduncle; more pronounced dorsal fin-anal fin setback, especially in females; and, adults are larger. In preserved material, markings on the ventral surface of adults, are typically a distinct v-shaped black mark originating in the isthmus that is often extended posteriorly by two parallel dotted lines to near the pelvic fins (more distinct in males) (Fig. 9b), also differentiates it from *G. toourtkoourt*. Less caudal fin and anal fin rays, one more longitudinal black lateral body stripe and less laterosensory pores in the preopercular-supramaxillary series differentiate it from *Galaxiella nigrostriata*. Differs from *Galaxiella munda* by being shorter overall, having less caudal and anal fin rays, more pectoral fin rays, less vertebrae, and possessing longitudinal dark lateral body stripes in adults.

The holotype has become partially dried, particularly anterior to the insertion of the pelvic fins. Therefore, morphometric and some meristic characters (e.g. gill rakers on upper limb of first gill arch; branched anal fin rays) could not be reliably elucidated. An old register card at NMV states that there were originally five paratypes, and that one was sent on exchange to E.O.G. Scott, Queen Victoria Museum, Launceston, on 14 May 1940. The paratype arrived at QVM (letter from E.O.G. Scott to G. Mack (NMV) dated 22 May 1940, seen; QVMAG Administrative Records), but its existence in the collection has not been able to be confirmed to date (David Maynard, *pers. comm.* August 2015).

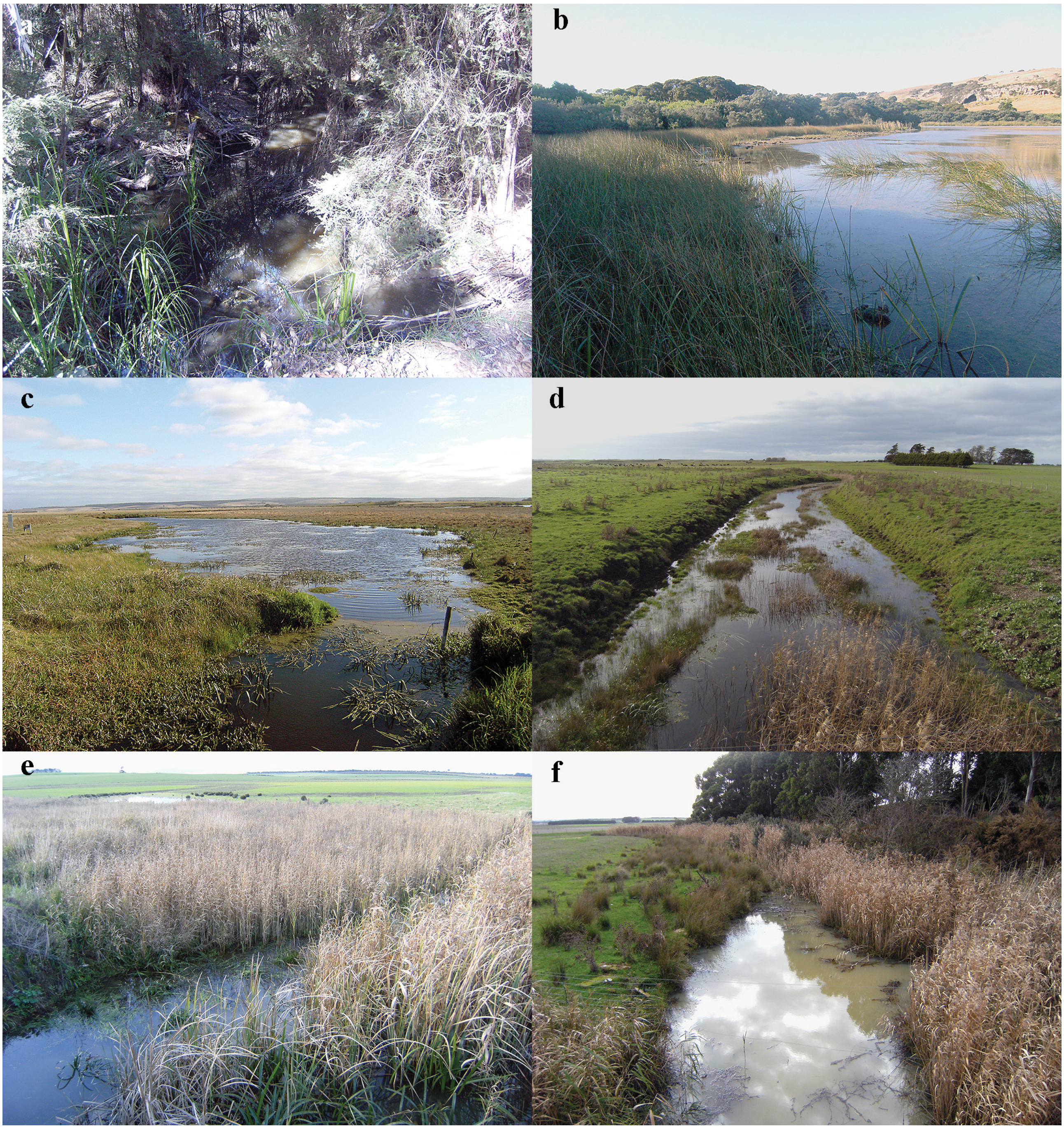


FIGURE 12. Examples of aquatic habitats occupied by *Galaxiella toourtkoourt*: a) Fitzroy River, T and W Road, Cobboboonee, Victoria, b) Bridgewater Lakes, Bridgwater Lakes Road, Tarragal, Victoria, c) Darlot Creek tributary, Etterick Tyrendarra Road, Tyrendarra, Victoria, d) Moyne River tributary, Woolsthorpe Heywood Road, Moyne, Victoria, e) Crawford River, Condah Coleraine Road, Branxholme, Victoria, f) Gosling Creek, Division Road, Murroon, Victoria.

There are small, additional morphological differences between this species and *G. toourtkoourt* that may be of secondary taxonomic importance: length of the caudal peduncle flanges, positioning of eye and colour in the wild (see Remarks section for *G. toourtkoourt*). There are also errors with respect to the number of preopercular-supermaxillary pores and gill raker morphology listed in McDowall & Frankenberg (1981) for this species, and for *Galaxiella* in general (see Discussion)

FIGURE 13. Examples of aquatic habitats occupied by *Galaxiella pusilla* s. s.: a) Dandenong Creek tributary, Brady Road, Endeavour Hills, Victoria, b) King Parrot Creek, Greenshield Road, Drouin, Victoria, c) Morwell River, Princes Freeway, Morwell, Victoria, d) Perry River, Princes Highway, Delvine, Victoria, e) Cobblers Creek, Forge Creek Road, Bairnsdale, Victoria, f) Big Waterhouse Lake, Homestead Road, Waterhouse, Tasmania.

Discussion

Morphometric and meristic differences support substantial genetic differentiation of eastern and western populations (Coleman *et al.* 2010; Unmack 2012; Coleman *et al.* 2013) that sufficiently warrant the separation of *G. pusilla* s.l. into two species. Accordingly, a new species, *G. toourtkoourt*, was described for the western lineage and *Galaxiella pusilla* s.s. was redescribed for the eastern lineage based on a greater number of specimens than previous descriptions by Mack (1936) and McDowall & Frankenberg (1981). Based on the recent update to the number of extant species in the Galaxiidae (Raadik 2014), our revision increases the number of species in the

Galaxiidae to 65, with 62 species in five genera in the Galaxiinae. It also increases the number of endemic species in this family in Australia to 34, of which 20 are endemic to the mainland. As well as the inclusion of *G. toourtkoourt*, several revisions were made to the previous taxonomic key to species of *Galaxiella* by McDowall & Frankenberg (1981).

In addition to geographic and genetic distinctness, important differences between *G. toourtkoourt* and *G. pusilla* s.s. include: the size of adults, number of vertebrae, length of caudal peduncle, dorsal fin-anal fin setback (most notably in females) and ventral surface markings in adults. A reduced dorsal fin-anal fin setback in *G. toourtkoourt* also distinguishes it from *G. nigrostriata* and *G. munda*, as do differences in body length, and the number of caudal and anal fin rays, vertebrae, longitudinal black stripes in adults and laterosensory pores in the preopercular-supramaxillary series.

Galaxiella pusilla s.l can be typically found in habitats that are shallow, have low flow velocities, are semi-permanent and contain dense stands of aquatic vegetation, including lakes, wetlands, swamps, billabongs, small creeks and earthen drains (e.g. Backhouse & Vanner 1978; McDowall & Frankenberg 1981; Beck 1985; Saddlier *et al.* 2010). During this study, common habitat features at sites where either *G. toourtkoourt* or *G. pusilla* s.s. occurred were consistent with those characteristics and also included, partial shading, a substratum dominated by fine clay and silt (occasionally sand) with deposits of coarse particulate organic matter, and dense vegetation comprising mostly emergent and submerged aquatic species. *Galaxiella toourtkoourt* and *G. pusilla* s.s. occur in a wide range of water quality conditions. Observed differences in habitats between *Galaxiella pusilla* s.s. and *G. toourtkoourt* include their salinity and elevational ranges; however, these results are based on field observations that may not accurately reflect tolerances associated with these factors.

From a conservation perspective, the formal recognition of *Galaxiella pusilla* and *G. toourtkoourt* as distinct taxa will enable independent management strategies to be tailored to account for their uniqueness and potential ecological differences, and is expected to lead to more successful outcomes for both species. For example, *G. pusilla* s.s. have much lower genetic diversity (Coleman *et al.* 2010; Coleman *et al.* 2013), are under substantial threat from habitat loss and habitat isolation from rapidly expanding urban areas in near Melbourne, and multiple surveys in 2008 and 2009 in northwest Tasmania and Flinders Island suggest that populations in these areas have dramatically contracted (Coleman *et al.* 2010; Coleman *et al.* 2013). Historical decline in distribution and area of occupancy is also indicated by a record of this species (NMV A.8330) from Scotchmans Creek in Yarra River catchment, Victoria, an area where extensive wetlands existed prior to urbanisation (e.g. Burns 1984; Presland 1994; Lacey 2004; Otto 2005).

In addition to the inclusion of *G. toourtkoourt*, we have made a number of revisions to the previous taxonomic key to species of *Galaxiella* by McDowall & Frankenberg (1981). Firstly, there are inconsistencies between their taxonomic key (pg. 552), descriptions (pg. 556, 562, 568) and illustrations (pg. 588) for all species regarding the number of pores in the preopercular-suborbital-supramaxillary series. For example, *G. pusilla* is listed with: 8 pores in the key (p. 552) and 8 pores in description (p. 556) but 7 pores in Fig. 47 (p. 588). Another inconsistency in McDowall & Frankenberg (1981) relates to gill raker morphology in the key, descriptions and illustrations (pg. 585) for all species. For example, gill rakers for *G. pusilla* and *G. nigrostriata* in the key are described as of 'moderate length', while in the species descriptions they are described as 'long and slender' (which matches more closely with Fig. 44 on pg. 585). Examination of *G. pusilla*, *G. toourtkoourt*, *G. nigrostriata* and *G. munda* specimens at NMV confirmed that the correct number of pores for all *Galaxiella* are depicted on pg. 588 of McDowall & Frankenberg (1981). On the other hand, examination of gill raker morphology on the same specimens did not reveal substantial differences between *Galaxiella* species. Accordingly, we have corrected the number of pores in our *Galaxiella* key and removed reference to differences in gill raker morphology. Frequency and range information for meristic characters has also been updated for *G. pusilla* s.s. and *G. toourtkoourt* in the taxonomic key, based on a much larger dataset in this study, and reference to body depth at vent/standard length has been removed due to strongly overlapping ranges between all species.

Acknowledgements

We thank Melbourne Water Corporation for funding this research. Special thanks to James Coleman for assistance with collecting specimens, to the Arthur Rylah Institute for Environmental Research (Victorian Department of Environment, Land, Water and Planning) for providing access to laboratory facilities during the study, and to

Martin Gomon (Museum Victoria) for use of the X-ray equipment. Thanks also to Dianne Bray (Museum Victoria) for specimen registration and access to registered specimens, Mark McGrouther (Australian Museum) and Ralph Forster (South Australian Museum) for paratype registration, David Maynard (Queen Victoria Museum) for collection details, Ross Smith (Queen Victoria Museum) for locating historical correspondence, and to Rhyll Plant for the scientific illustrations. We are grateful to Denis Rose, Ben Church (Gunditj Mirring Traditional Owners Aboriginal Corporation) and Joel Wright (Victorian Aboriginal Corporation for Languages) who helped identify an appropriate indigenous name for the new *Galaxiella* species. Three anonymous reviewers are thanked for comments that helped substantially improve this paper. Michael Hammer and Rudie Kuiter kindly provided images of fish to use in this manuscript. Research was carried out with The University of Melbourne animal ethics approval (AEC 1312848.1) and State of Victoria research permits for threatened species and national parks (DEPI 10006773), and fisheries (DEPI RP1147).

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APPENDIX 1. One-way ANOVA tests for differences in size corrected morphometric characters between eastern and western regions. Significant results after Bonferroni correction are in bold.

Character	Females		Males	
	F value	*P* value	*F* value	*P* value
Body depth at vent	3.04	0.084	12.84	**<0.001**
Caudal peduncle length	76.28	**<0.001**	84.30	**<0.001**
Caudal peduncle depth	2.95	0.088	0.58	0.448
Caudal fin length	5.78	0.018	0.08	0.775
Pre-dorsal fin length	0.44	0.509	1.32	0.254
Pre-anal fin length	36.18	**<0.001**	27.76	**<0.001**
Dorsal fin to anal fin setback	79.03	**<0.001**	29.08	**<0.001**
Dorsal fin base length	0.62	0.433	1.43	0.234
Dorsal fin length	1.21	0.273	<0.01	0.971
Anal fin length	1.06	0.305	2.04	0.157
Pectoral fin length	6.19	0.014	0.45	0.503
Pelvic fin length	30.21	**<0.001**	8.89	0.004
Pre-pelvic fin length	46.90	**<0.001**	31.00	**<0.001**
Distance between pelvic and anal fin bases	2.65	0.106	1.02	0.316
Head width	60.20	**<0.001**	31.07	**<0.001**
Post-orbital head length	20.47	**<0.001**	19.81	**<0.001**
Inter-orbital width	0.52	0.474	0.18	0.671
Eye diameter	7.46	0.007	1.58	0.211
Upper jaw length	38.56	**<0.001**	17.89	**<0.001**
Gape width	96.66	**<0.001**	77.67	**<0.001**
Gape depth	19.96	**<0.001**	7.76	0.006
Keel length	1.02	0.315	0.66	0.420
Keel depth	26.46	**<0.001**	12.53	**<0.001**

APPENDIX 2. Summary statistics from habitat assessments at sites where *Galaxiella pusilla* s.s. ('east') and *Galaxiella toourtkoourt* ('west') were collected between 2007-2010.

	Temperature (^{o}C)		Dissolved oxygen (%)		pH		Conductivity (µS)		Turbidity (NTU)		Elevation (m)		Shading (%)		Maximum water depth (m)	
	East	West	East	West	East	West	East	West	East	West	East	West	East	West	East	West
n	52	49	36	49	52	49	52	48	23	41	50	51	52	51	52	51
Minimum	5.8	5.2	18.2	20.0	5.0	5.3	36	94	1.0	1.0	8	7	0.0	0.0	0.1	0.1
25th percentile	11.0	13.8	44.5	50.3	6.4	7.2	267	1013	6.0	3.8	25	22	10.0	5.0	0.5	0.5
Mean	14.8	16.3	56.9	81.3	6.6	7.6	708	2455	26.6	15.7	54	104	35.9	27.0	1.1	1.1
SE mean	0.7	0.8	3.8	6.1	0.1	0.1	88	377	7.1	2.7	4	13	3.7	4.5	0.1	0.1
Median	14.3	16.8	55.1	77.1	6.6	7.8	487	1630	11.5	11.0	58	69	30.0	10.0	1.0	1.0
75th percentile	19.6	20.5	68.3	96.1	7.0	8.2	959	2980	29.3	22.9	72	176	60.0	50.0	1.5	2.0
maximum	24.8	26.9	130.0	263.0	7.8	9.3	3070	13620	133.0	96.0	111	376	80.0	100.0	>2.0	>2.0

	Clay (%)		Silt (%)		Sand (%)		Gravel (%)		Pebble (%)		Cobble (%)		Boulder (%)		CPOM (%)		LWD (%)	
	East	West	East	West	East	West	East	West	East	West	East	West	East	West	East	West	East	West
n	52	51	52	51	52	51	52	51	52	51	52	51	52	51	52	51	52	51
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25th percentile	10.0	0.0	30.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0
Mean	32.9	33.8	42.7	43.0	20.7	17.5	3.3	2.5	0.2	2.1	0.0	2.2	0.4	2.5	30.6	25.0	3.6	1.9
SE mean	3.3	3.9	3.0	3.7	4.2	4.0	1.3	1.2	0.2	1.0	0.0	1.1	0.3	1.7	4.1	4.7	0.6	0.4
Median	42.5	40.0	50.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	10.0	5.0	0.0
75th percentile	50.0	50.0	50.0	50.0	34.0	22.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	40.0	5.0	5.0
maximum	80.0	100.0	100.0	100.0	100.0	100.0	40.0	45.0	10.0	45.0	0.0	50.0	10.0	75.0	90.0	100.0	20.0	10.0

	Emergent vegetation (%)		Floating vegetation (%)		Submerged vegetation (%)		Terrestrial exotic vegetation (%)		Terrestrial native vegetation (%)	
	East	West	East	West	East	West	East	West	East	West
n	52	51	52	51	52	51	52	51	52	51
Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25th percentile	23.8	15.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0
Mean	45.0	35.8	11.2	3.2	12.3	17.2	1.4	1.2	17.1	7.3
SE mean	4.3	4.0	3.0	1.3	2.9	3.5	0.4	0.4	2.2	1.7
Median	35.0	25.0	0.0	0.0	0.0	5.0	0.0	0.0	15.0	0.0
75th percentile	72.5	50.0	10.0	0.0	12.5	22.5	0.0	0.0	21.3	10.0
maximum	100.0	100.0	80.0	50.0	75.0	90.0	10.0	15.0	65.0	60.0